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TOXICITY TO AQUATIC ORGANISMS AND CHEMISTRY OF NINE SELECTED WATERBORNE POLLUTANTS FROM MUNITIONS MANUFACTURE-A LITERATURE EVALUATION

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Fort Detrick, Maryland

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Dinitrotoluene	Pentaerythritol-tetranitrate	Potassium perchlorate	Tetryl
Lead styphnate		Red phosphorus	Trinitroresorcinol
			Wastewater

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The literature regarding the toxicity to aquatic organisms and the related chemistry of nine military-relevant compounds was evaluated and reviewed. The selected compounds, munitions manufacturing products of potential concern as water-borne pollutants, are dinitrotoluene, tetryl and picric acid, trinitroresorcinol and lead styphnate, pentaerythritol tetranitrate, nitroguanidine, tetracene, potassium perchlorate, red phosphorus and strontium salts.

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Based on the available toxicity data, recommendations were made for further investigations to be conducted on the first seven of the listed compounds. No further toxicity studies have been proposed for red phosphorus and the strontium compounds. The required further work includes the determination of the 96-hour TL_m using both native freshwater and marine target species; the conduct of complete chronic bioassay testing over the life-cycle of the same organisms used in the acute tests; the determination of the rates and products of microbial degradation, especially in the wastewaters; and complete wastewater analysis to estimate the concentrations of the pollutants and their photolysis and/or degradation products.

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INTRODUCTION

The objective of this work is to provide part of a data base for defining standards for military-unique product discharges into waters subject to state and federal regulations. This report is a continuation of earlier work initiated to provide a summary review and evaluation of the toxicological and related literature of military munitions production products of concern as waterborne pollutants. The first report (Dacre and Rosenblatt, 1974)[1] considered both the mammalian toxicology and toxicity to aquatic organisms of four munitions pollutants - nitrocellulose, nitroglycerin, white phosphorus and trinitrotoluene. The present report deals with the toxicology and chemistry of a further nine munitions pollutants, but only as related to aquatic organisms.

The mammalian toxicology of six of the nine compounds [dinitrotoluene (DNT), lead styphnate, pentaerythritol tetranitrate (PETN), nitroguanidine, tetracene and red phosphorus] has recently been evaluated in a report by Tardiff and Mullaney (1974)[2].

Basis for Selection of Pollutants. The nine waterborne pollutants were selected from the list furnished to the Office of the Surgeon General, US Army, by the US Army Materiel Command (see Rosenblatt, Small and Barkley, 1973; Small and Rosenblatt, 1974)[3,4]. All but one of the compounds are listed in Gategory 1 (see Table 1 of the latter report), i.e., compounds requiring additional research before standards can be recommended. The ninth compound, tetryl, was included on the basis of the large amounts estimated to be present in the soil at Joliet Army Ammunition Plant (see Small and Rosenblatt, 1974)[4].

The chemistry, analysis, production, uses, environmental fate and concentration levels of all of the pollutants with the exception of strontium nitrate form the basis of the report by Small and Rosenblatt (1974)[4]. Some aspects of mammalian and aquatic toxicology of DNT as related to TNT wastewaters, are included in the report by Dacre and Rosenblatt (1974)[1].

Approach. Each succeeding section of this report deals with one of the nine selected pollutants, more or less in order of priority. For each compound the published literature surveyed has been summarized in the introductory approach to the toxicity to aquatic organisms; the toxicity, including biotransformations if known; aquatic chemistry and analysis, and recommendations for future work, has been reviewed. At the end of the report, there is a summary of all the research recommendations for the pollutants considered.

DINITROTOLUENE (DNT)

Approach to Toxicity to Aquatic Organisms. The published literature has been surveyed through July 1974 by means of *Chemical Abstracts*, using the survey scheme presented in Table 1. For Volumes 1-78 (1907-1973), the appropriate index headings were surveyed; for Volumes 79-80 (1973-1974), the keyword citations were surveyed issue by issue.

Table 1. Literature Survey Scheme for DNT

Citation	Chemical Abstracts Vol.						
	80	79	78	77	76	75-66	65-1
Dinitrotoluene	X	X				X	X
Toluene, dinitro (all isomers)	X	X				X	X
Benzene, methyldinitro (all isomers)	X	X	X	X	X		

Toxicity to Aquatic Organisms. For an unspecified isomer or mixture of isomers of DNT, Burton (1971)[5] reports 24, 48 and 96-hour TLm values of 50, 27 and 16 mg/l, resp., for bluegills (*Lepomis macrochirus*) maintained at 20°C. On the basis of Warburg studies, Randall and King (1971)[6] concluded that DNT is relatively nontoxic to microorganisms, but is slowly biodegraded. Bringmann and Kuhn (1971)[7] on the other hand, found that 2,4- and 2,6-DNT at the 100-150 mg/l level were almost completely removed by two stage biological oxidation. The first stage, which removed 80-90%, utilized *Azotobacter agilis*, while the second stage was seeded with conventional activated sludge. These results are not necessarily in conflict with the Warburg studies, only a moderate volume of oxygen is required to convert dinitrotoluene to carbon dioxide and ammonia, and it is probable that some of the material is removed by absorption.

$$C_7H_6N_2O_4 + 5O_2 \longrightarrow 7CO_2 + 2NH_3$$

DNT is moderately fungistatic at 200 mg/l against seven common fungi, as shown in Table 2 (Zsolnai, 1961)[8]. Against the mold

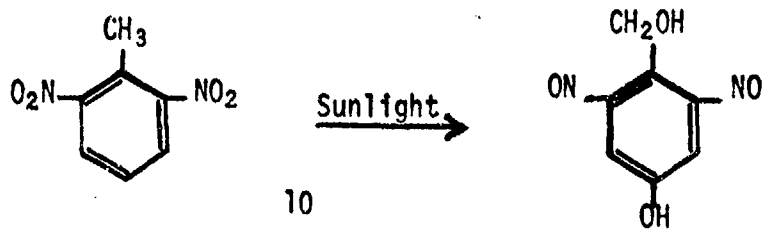
Trichoderma viride 2,4-DNT at a level of 115 mg/l brings about 50% inhibition of growth (Simon and Blackman, 1953)[9]. Galuzova (1963)[10] claims that DNT (unspecified isomer) at 10 mg/l affect nitrification and "ammonification"; he also suggests a maximum permissible concentration of 0.5 mg/l in reservoirs on the basis of the taste detection threshold.

Table 2. Fungistatic Action of DNT at 200 mg/l (Zsolnai, 1961)[8]

Fungi	Action of 2,4-DNT	Action of 3,5-DNT
Penicillium simplicissimum	Inhibition	Inhibition
Aspergillus niger	Inhibition	Partial inhibition
Trichothecium roseum	Inhibition	Inhibition
Candida albicans	Partial inhibition	No effect
Achorion quinckeanum	Inhibition	Inhibition
Trichophyton gypseum	Inhibition	Inhibition
Eidermophyton Kaufman-Wolff	Inhibition	Inhibition

Chemistry of DNT. The chemistry of manufacture of DNT has been reviewed by Small and Rosenblatt (1974)[4]. There are six possible isomers of dinitrotoluene, of which the 2,4-isomer (75%) and 2,6-isomer (20%) predominate. DNT is also present in wastewaters from TNT manufacture (Dacre and Rosenblatt, 1974)[1].

Schultz and Ganguly (1925)[11] have investigated the reactions of powdered polynitrotoluenes in the presence of sunlight. 2,6-Dinitrotoluene undergoes internal oxidation-reduction to produce a material identified as 2,6-dinitroso-4-hydroxybenzyl alcohol, while 2,4-dinitrotoluene is unchanged. It would be of interest to learn whether 2,6-DNT dissolved in water undergoes the same reaction; it is known that aqueous DNT solutions turn yellow when exposed to sunlight.



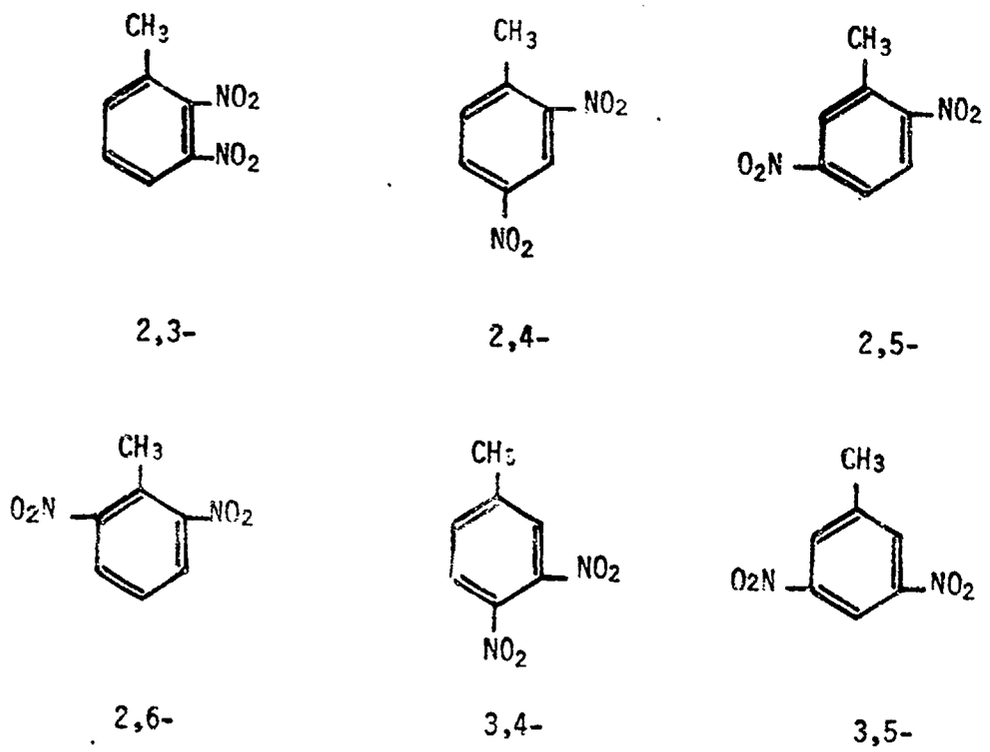


Figure 1. Structural Isomers of DNT

Analysis of DNT. Although a procedure for trace analysis of DNT isomers in wastewater has not been reported, gas-liquid chromatography (glc) appears promising. Parsons, *et al.* (1961)[12] have succeeded in separating and identifying the six isomers of DNT on an Apiezon column, using a thermal conductivity detector (TCD). Separation and detection of 4 to 5 isomers have been achieved using a silicone column with TCD (Camera, *et al.*, 1965)[13] or flame ionization detector (Etienne and Tranchant, 1965; Alley and Dykes, 1972)[14,15]. A Russian paper (Zatkovetskii, *et al.*, 1972)[16] describes a glc procedure for estimation of DNT in wastewater, with an accuracy of ± 5% "at a material content as low as 5 mg/l". Electron capture detectors appear not to have been exploited.

Several papers report the separation of DNT from other explosives by paper (Krien, 1963)[17] and thin-layer chromatography (tlc) (Franck-Neumann and Jossang, 1964; Bohm, 1966)[18,19], and one describes separation of the 2,4- and 2,6-isomers (Prat and Forestier, 1962)[20].

Engelbertz and Babel (1957)[21] have devised a colorimetric procedure for detection and estimation of five DNT isomers in biological fluids. The ethylenediamine adduct is extracted with an organic solvent and determined photometrically with apparent detection limits of 0.1-5 mg/l (0.2 mg/l for the 2,4-isomer). Photometric methods have been described for estimation of the colored products formed from 2,4- or 2,6-DNT and acetone in the presence of alkali (Baernstein, 1943; Kaminskii, *et al.*, 1966)[22,23]. The precision appears to be about + 5 mg/l. Techniques involving chemical reduction of nitro groups (Selig, 1961)[24], infrared spectroscopy (Tanaka and Kinoshita, 1960)[25], and polarography (Bourjol, *et al.*, 1954)[26] have been devised, but none has been applied to the problem of wastewater analysis.

A glc separation of 5 of the isomers of dinitrotoluene was achieved using a 4' U-tube glass column containing 5% Carbowax 20M on CHROMOSORB W (AW), 60/80 mesh, at 150°C and helium flow of 75 cm^3/min (Dennis, 1972 - unpublished results). The isomers separated in the following manner.

Compound	Retention Time (min)
2,6-DNT	9.5
2,5-DNT	11.0
2,4-DNT	13.0
2,3-DNT	16.5
3,4-DNT	26.0

Recommendations for Further Work.

1. The wastewaters of concern should be analyzed to estimate the concentrations of each isomer of DNT. The environmental chemistry of the separate isomers should be studied to establish the rates and products of conversion effected in water by sunlight under natural conditions of pH and temperature. The rates and products of microbial degradation of significant DNT isomers (mainly 2,4- and 2,6-DNT) should be examined. The intent of these studies is to determine the rate of disappearance of DNT from receiving waters, and to assess the likelihood of build-up of possibly hazardous degradation products.

2. The acute toxicities of the separate isomers of DNT and any important degradation product should be measured for appropriate freshwater and marine target species, such as bluegill (*Lepomis macrochirus*), common killifish (*Fundulus heteroclitus*) and *Daphnia*. Acute toxicities should also be measured for the DNT wastewaters of concern, and the same wastewaters after prolonged exposure to sunlight. Chronic toxicity studies should be carried out over the life cycle of organisms used in acute studies for DNT isomers and important degradation products shown to be present in the wastewaters. Chronic toxicities should also be examined for the wastewaters themselves and for the photolyzed wastewaters.

3. The build-up of DNT in fish, algae and a few other aquatic organisms should be examined using the highest levels of DNT isomers likely to be present in wastewaters.

TETRYL AND PICRIC ACID

Approach to Toxicity to Aquatic Organisms. A survey of the literature through July, 1974, revealed no data concerning the toxicity of tetryl to aquatic organisms, except for a single paper on the toxicity of tetryl wastewaters to minnows (Degani, 1943)[27]. The survey scheme for *Chemical Abstracts* is presented in Table 3. For Volumes 1-78 (1907-1973), the appropriate index headings were surveyed; for Volumes 79-80 (1973-1974), the appropriate keyword citations were surveyed issue by issue. Secondary sources, primarily studies on mammalian toxicology; provided no aquatic references.

Because picric acid may be a significant component of tetryl wastewaters, a literature survey of the toxicity of picric acid to aquatic organisms has been conducted as part of this study. The survey scheme for *Chemical Abstracts* is presented in Table 4. For Volumes 1-78 (1907-1973), the appropriate index headings were surveyed; for Volumes 79-80 (1973-1974), the keyword citations were surveyed issue by issue. Many useful references are provided by McKee and Wolf (1963)[28]. Because picramic acid is an important metabolite of picric acid, the aquatic toxicity of this compound was also surveyed, as shown in Table 4.

Toxicity of Picric Acid to Aquatic Organisms. Two papers describe the toxicity of picric acid to fish, both from the Water Pollution

Table 3. Literature Survey Scheme for Tetryl

Citation	Chemical Abstracts Vol. 80	79	78	77	76	75-66	65-56	55-51	50-1
Tetryl	X	X				X	X	X	X
Nitramine	X	X				X	X	X	X
Aniline, N-methyl-N,2,4,6-tetranitro	X	X		X	X	X	X	X	X
Benzeneamine, N-methyl-N,2,4,6-tetranitro	X	X	X						
Tetranitrophenyl-methylamine	X	X				X	X	X	X
Trinitrophenyl methylnitramine	X	X				X	X	X	X

Table 4. Literature Survey Scheme for Picric Acid and Picramic Acid

Citation	Chemical Abstracts Vol. 80	79	78	77	76	75-66	65-56	55-51	50-1
Picric acid	X	X	X	X	X	X	X	X	X
Picrate						X	X	X	X
Phenol, 2,4,6-trinitro	X	X	X						
Picramic acid	X	X						X	X
Phenol, 2-amino-4,6-dinitro			X	X	X	X	X		

Research Laboratory, Watford, England. Wilkinson (1951)[29] reports that the average toxicity to minnows (*Phoxinus phoxinus*), measured as the reciprocal of overturning time (i.e., the time in which the fish turns on its side), is linearly proportional to the concentration of

picric acid. The overturning time is about 200 minutes at 1400 mg/l, and roughly 1 day at 100 mg/l. The pH of the test water was not specified. This is an important consideration, because trinitrophenol is a strong acid, and the toxicity could be related in part to a low pH. Grindley (1946)[30] considered the limiting concentration (minimum toxic level) of sodium picrate to be about 30 mg/l for *Phoxinus*. Both authors agree that dinitrophenol (or its sodium salt) is far more toxic than picric acid to minnows. Grindley's data, summarized in Table 5, are in general agreement with Wilkinson's data, indicating that differences in pH were not important. Lindahl and Marcstrom (1958)[31] report an ambivalent response to picric acid by the roach, *Leuciscus rutilus*, which is attracted at low levels, but is repelled, with signs of distress, at 65.7 mg/l.

Table 5. Toxicity of Sodium Picrate to Minnows (Grindley, 1946)[30]

Sodium Picrate Conc. mg/l	Temp. °C	Dissolved Oxygen Init., mg/l	pH Init.	Mean Overturning Time, min.	No. Fish
2000	16.7-17.2	9.9	7.3	192	6
1500	16.8	9.2	---	237	6
1000	14.8-17.9	---	---	369	6
700	16.4-16.7	10.5	7.7	474	6
400	13.0-17.1	---	7.9	826	6
300	13.3-16.7	---	7.9	1124	6
200	13.0-20.0	9.2	7.8	1563	6

Concerning the effect of picric acid on macroinvertebrates, LeFevre (1945, 1948)[32,33] has noted that activation of the eggs of the marine polychaete *Nereis limbata* (clanworm) is inhibited by picric acid at the 230 mg/l level, but with concurrent sensitization for subsequent stimulation. For cysts of the root nematode *Heterodera*, on the other hand, picric acid at 100-200 mg/l serves as a hatching agent (Fenwick, 1943; Clarke and Shepherd, 1964)[34,35] and larval stimulant (Nolte, 1955)[36], substituting for the natural agents present in root excretions. These findings suggest the possibility that development

of benthic communities could be altered in subtle ways by picric acid at substantially lower levels. For the crustacean *Daphnia*, Bringmann and Kuhn (1959)[37] report a toxic threshold of 88 mg/l after 48 hours at 23°C.

Toxicity of Picric Acid to Microorganisms. Two papers report the toxicity of picric acid to amoebae. According to Pollack (1927)[38], a concentration of 1% is "extremely toxic" to an unspecified amoeba if applied to the surface, but is not toxic when injected in amounts equal to half the cell volume. Hajra (1959)[39] reports that *Neglaria gruberi* and *Acanthamoeba sp.* exhibit toxic responses at substantially different concentrations; 10 mg/l is half-lethal for *Acanthamoeba sp.*, but is well resisted by *N. gruberi*. For the cysts of either the LD_{50} is 2000 mg/l. For neither of these studies is the pH reported. For the protozoan *Microregma heterostoma*, Bringmann and Kuhn (1959)[40] found the 96 hour toxicity threshold to be 900 mg/l.

Bacteria are more resistant to picric acid. For example, the five-day BOD of domestic sewage is not lowered significantly by picric acid at the 100 mg/l level, and is lowered only 36% at 1000 mg/l (Ruchhoft and Norris, 1946)[41]. Wilkinson (1951)[29] has found that the presence of picric acid and dinitrophenol in wastewater (conc. unstated, but presumably 10-100 mg/l) causes an increase in population of bacteria. Cooper and Mason (1927, 1928)[42,43] have investigated the bactericidal action of picric acid in some detail, as shown in Table 6. Demerec, *et al.* (1951)[44] found that the number of mutations exhibited by *Escherichia coli* exposed to 100-180 mg/l of picric acid exceeded the controls by 10-fold at most. The experimental pH is not explicitly provided in most of these papers, but Cowles and Klotz (1948)[45] have shown that the toxicity of picric acid and a number of other nitrophenols to *E. coli* and *Bacillus mesentericus* increases many fold as the pH is diminished from pH 8.5 to 5.5. Bringmann and Kuhn (1959)[37] report that 1000 mg/l is not toxic for *E. coli*. Lakchaura and Jagger (1972)[46] have found that posttreatment with picric acid at a level of 345 mg/l actually enhances the survival of *E. coli* cells subjected to far-ultraviolet (254 nm) irradiation.

Several papers establish that the fungicidal properties of phenol are enhanced by the presence of nitro groups (Plantefol, 1922; Simon and Blackman, 1952; Galanti, 1959; Zsolnai, 1961; Polster, 1967)[47,48,49,50,51]. However, picric acid is only mildly fungistatic in comparison with dinitrophenol. Plantefol (1922)[47] found that 400 mg/l of picric acid prevents germination of *Sterigmatocystis nigra*, while Zsolnai (1961)[50]

Table 6. Toxicity of Picric Acid to Bacteria

Bacteria	Picric Acid Conc. mg/l (pH)	Temp. °C	Exposure Period hrs.	Stated Effect	Reference
Escherichia coli	1330	37	48	Inhibitory[a]	Cooper and Mason, 1928, 1928[42 43]
Pseudomonas fluoresceus	2100	37	48	Inhibitory[a]	Cooper and Mason, 1927[42]
Pseudomonas fluoresceus	3330	37	48	Inhibitory[a]	Cooper and Mason, 1928[43]
Serratia marcescens	2860	37	48	Inhibitory[a]	Cooper and Mason, 1928[43]
Bacillus mesentericus	1820	37	48	Inhibitory[a]	Cooper and Mason, 1928[43]
Bacillus mesentericus	300 (5.5)	37	96	Inhibitory[a]	Cowles and Klotz, 1948[45]
Bacillus mesentericus	3200 (8.5)	37	96	Inhibitory[a]	Cowles and Klotz, 1948[45]
Unsp. thermophiles	2220	53	24	Inhibitory[a]	Cooper and Mason, 1928[43]
Pseudomonas non-liquefaciens	200	20	1/2-2	Germicidal[b]	Cooper and Mason, 1928[43]
Pseudomonas non-liquefaciens	200	37	1/2	Germicidal[b]	Cooper and Mason, 1928[43]
Pseudomonas non-liquefaciens	125	37	2	Germicidal[b]	Cooper and Mason, 1928[43]

Table 6. Toxicity of Picric Acid to Bacteria (Cont)

Bacteria	Picric Acid Conc. mg/l (pH)	Temp. °C	Exposure Period hrs.	Stated Effect	Reference
Pseudomonas non-liquefaciens	>3330	37	48	Inhibitory[a]	Cooper and Mason, 1928[43]
Escherichia coli	1110	20	1/2	Germicidal[b]	Cooper and Mason, 1928[43]
Escherichia coli	570	20	2	Germicidal[b]	Cooper and Mason, 1928[43]
Escherichia coli	285	37	1/2	Germicidal[b]	Cooper and Mason, 1928[43]
Escherichia coli	200	37	2	Germicidal[b]	Cooper and Mason, 1928[43]
Escherichia coli	170-180	37	24	2-35% survival	Demerec, *et al.*, 1951[44]
Escherichia coli	800 (5.5)	37	96	Inhibitory	Cowles and Klotz, 1948[45]
Escherichia coli	3200 (8.5)	37	96	Inhibitory	Cowles and Klotz, 1948[45]
Escherichia coli	1000	--	--	not toxic	Bringmann and Kuhn, 1959[37]

[a]Apparently buffered

[b]Apparently unbuffered

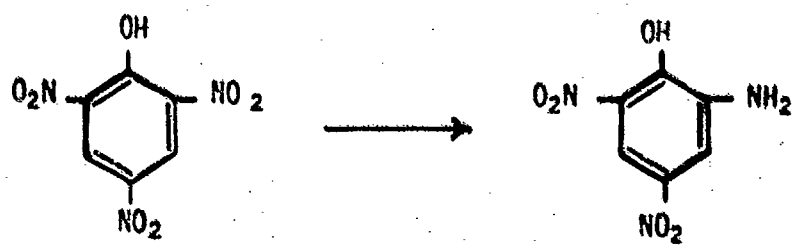
has shown that 200 mg/l is not fungicidal to *Penicillium simplicissimum, Aspergillus niger, Tricothecium roseum, Candida albicans, Achorion quinckeanum, Trichyphyton gypseum, Epidermophyton* Kaufman-Wolff, or *Saccharomyces cerevisiae*. Galanti (1959)[49] has reported that very low picric acid levels (0.2-3 mg/l) actually promote the production of itaconic acid by *Aspergillus terreus*. In studies by Simon and Blackman (1952)[48], 300 mg/l was required to halve the respiration rate of yeast at pH 3. The authors noted that impurities in picric acid, probably dinitrophenol, may have been responsible for the toxicity attributed to picric acid in earlier reports.

Vella and Zampieri (1950)[52] have examined the antiviral action of picric acid. A 10% solution was found to inhibit one species of *Mycobacterium* phage.

Phytotoxicity of Picric Acid. Simon and Blackman (1952)[48] found that 1750 mg/l of purified picric acid was required to halve the growth rate of *Lemna minor* grown in water culture at pH 5.1.

For the green alga *Scenedesmus quadricauda* the 96-hour toxicity threshold is said by Bringmann and Kuhn (1959)[37] to occur at 240 mg/l at 24°C.

Biotransformation and Subcellular Effects of Picric Acid. In early studies (Barral, 1915; Guerbet and Mayer, 1932; Giorgi, 1924)[53,54,55] elimination of picric acid from humans in the form of picramic acid was observed. In perfusion experiments with liver, kidney and spleen,



the liver exhibited the strongest reduction of picric acid (Giorgi, 1924)[55]. More recently, Tsukamura (1954, 1954, 1960)[56,57,58] showed that this conversion is effected by resting cells and cell-free extracts of

Mycobacterium tuberculosis avium. Gundersen and Jensen (1956)[59] report that an atypical strain of *Corynebacterium simplex* decomposed picric acid and some other nitrophenols to produce nitrites, indicating an alternative metabolic pathway. Chambers, *et al.* (1963)[60] found that phenol-adapted bacteria exposed to picric acid at a 100 mg/l level showed little oxygen uptake beyond that required for endogenous respiration. Although the authors concluded thereby that picric acid is resistant to oxidative degradation, it should be pointed out that picric acid incorporates in its formula most of the oxygen required for its conversion to carbon dioxide and ammonia. Thus, the manometric technique is poorly suited for monitoring oxidative biodegradation of picric acid.

$$C_6H_3N_3O_7 + 3H_2O + O_2 \longrightarrow 6CO_2 + 3NH_3$$

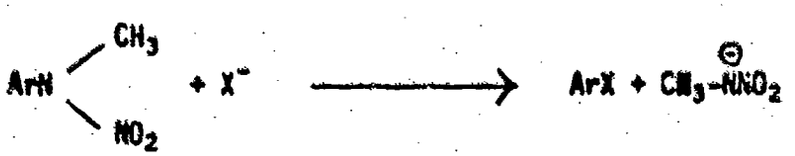
Picric acid has been found to be weakly inhibitory or noninhibitory for some enzyme systems, as shown in Table 7. According to a review by Weinbach and Garbus (1969)[61] picric acid, unlike 2,4-dinitrophenol, does not uncouple oxidative phosphorylation in isolated mitrochondria. Gladtke and Liss (1958)[62] suggest that because picric acid is always present at moderate pH as the picrate ion rather than as a fat-soluble molecule, it may never penetrate the mitochondria. Whatever the degree, inhibition of enzymes by picric acid appears to be noncompetitive.

Table 7. Inhibition of Enzymes by Picric Acid

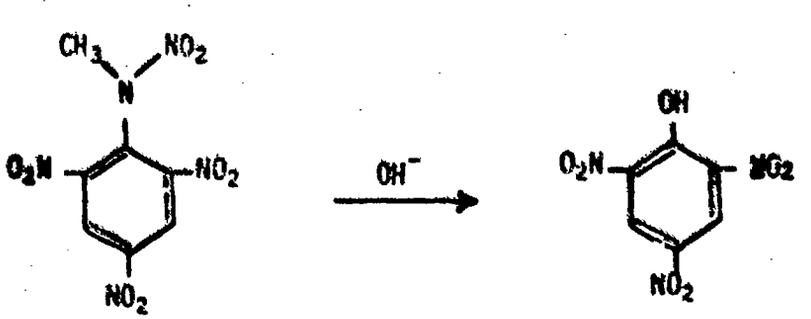
Enzyme System	Picric Acid Conc. mg/l	Reported Effect	Reference
Dog liver phosphatase	?	50% inhibition	Sanchez and Castella, 1950[63]
E. coli menadione reductase	7	38% inhibition	Mosllait and Nason, 1954[64]
E. coli quinone reductase	7	22% inhibition	Mosllait and Nason, 1954[64]
Rat liver nitrochondria (phosphorylase)	69	no inhibition	Gladtke and Liss, 1958[62]
Pig heart malate dehydrogenase	?	noncompetitive	Wedding, *et al.* 1967[65]

Toxicity of Picramic Acid. Because picramic acid is a common product of picrate metabolism, it seems advisable to review its toxicity, even though picramic acid has not been identified in the wastewaters of tetryl manufacture. There is very little data covering the nonmammalian toxicology of picramic acid, and what data there is points to a level of toxicity not significantly greater than that of picric acid. As an antibacterial agent it inhibits the growth of *Bacillus mesentericus* and *E. coli* at 110 mg/l (pH 5.5) to 4000 mg/l (pH 8.5) (Cowles and Klotz, 1948)[45] as well as the production of the hemolytic toxin streptolysin by *Streptococcus hemolyticus* (Himeno, 1954)[66], and *S. pyogenes* (Ito, *et al.*, 1948)[67]. As a fungicide it is inactive against eight common species at a level of 200 mg/l (Zsolnai, 1961)[50]. As a virucide it partly inhibits poliomyelitis virus (Kramer, *et al.*, 1955)[68] and *E. coli* T2r+ phage (Bourke, *et al.*, 1952)[69]. Picramic acid is more effective in uncoupling rat liver mitochondrial phosphorylation than picric acid; concentrations of 9 and 22 mg/l bring about rate reductions of 25% and 75%, respectively (Leader and Whitehouse, 1966)[70]. The toxicity of picramic acid, like that of picric acid and other strongly acidic phenols, is apparently greatest for the undissociated molecule (Fujita, 1966)[71].

Chemistry of Tetryl. The chemical properties of tetryl have been reviewed by Small and Rosenblatt (1974)[4]. There is evidence that certain negatively ring-substituted aromatic nitramines undergo nucleophilic displacement of the nitramine group (VanDuin, 1919; Urbansky, 1967)[72,73].



Thus, under basic conditions picric acid may be an important constituent of wastewaters containing tetryl. In fact, Hoffsommer and Rosen (1973)[74] have shown that tetryl dissolved in seawater at pH 8.1 and 25°C is largely converted to picric acid in a few months.



Analysis of Tetryl and Picric Acid. A highly sensitive procedure for determination of tetryl in seawater has been developed by Hoffsommer and Rosen (1972)[75]. The evaporated benzene extract of the seawater sample is analyzed by gas-liquid chromatography, using a ^{63}Ni electron capture detector and o-dinitrobenzene as internal standard. The authors claim a detection limit of about 0.02 µg/l. No important interferences were present in seawater stored in all-glass containers. Other analytical schemes have been principally concerned with analysis of explosive mixtures containing tetryl. These have employed paper chromatography (Krien, 1963)[17], tlc (Franck-Neumann and Jossang, 1964; Bohm, 1966; Prat and Forestier, 1963)[18,19,20], and two-dimensional tlc (Yasuda, 1970)[76]. Using p-diethylaminobenzaldehyde as spray reagent, 1 µg is about the least quantity of tetryl detectable. A quantitative procedure based on tlc has been reported by Hansson (1963)[77], who claims a detection limit of ± 1 µg.

Methods for the determination of picric acid have been reviewed by Hayashi, *et al.* (1965)[78]. These authors have devised a procedure for estimating picric acid in water solution by extraction into nitrobenzene with the metal chelate cation prepared by combination of 2,2'-dipyridyl and ferrous ammonium sulfate. The dipyridyl iron (II) picrate is measured spectrophotometrically. The detection limit appears to be about 0.5 mg picrate per liter of water. Interference from dinitrophenols can be eliminated by pH adjustment. Extraction of the picrate anion into an organic solvent for spectrophotometric determination has also been achieved using Violet Red in benzene (Koremman, *et al.*, 1968)[79]. A sensitivity of about 0.1 mg/l is claimed. In a related (but probably more tedious) procedure, picrate is determined volumetrically by titration with methylene blue in the presence of chloroform (Bolliger, 1939)[80]. Direct spectrophotometric estimation of ammonium picrate in concns >1 mg/l has been reported (Ruchhoft and Norris, 1946)[81]. The authors state that the method is suitable for wastewaters from shell loading plants, although interferences were not completely researched.

Tlc procedures have been described (Hansson, 1963; Parihar, *et al.*, 1968; Thielemann, 1971)[77,81,82], but have not been adapted for trace analysis of picric acid in water. A paper chromatographic technique said to give 5-10% precision for 2-6 µg samples of picric acid has been described (Perpar, *et al.*, 1959)[83]. For wastewater samples, both paper and thin-layer procedures require extraction of the picric acid from water, chromatography, elution of the isolated picric acid, and spectrophotometric analysis of the eluate. Although more tedious than direct spectrophotometric examination, interferences are minimized.

Chemical reduction of picric acid has been carried out quantitatively using chromium II (Tandon, 1959)[84] and vanadium II (Witry-Schwachtigen, 1955)[85] salts in the presence of suitable redox indicators. For the chromium II procedure the precision is reported to be better than ± 1 mg/l. However, interference can be expected from any other nitro compounds. Electrolytic methods of reduction of nitro groups have also been investigated. Polarography (Neiman, *et al.*, 1949; Ashworth and Walisch, 1961; Tur'yan and Zaitsev, 1962)[86,87,88], coulometry (Meites and Meites, 1956)[89] and electrolysis (Tur'yan, 1955)[90] have been reported. A single report implies a detection limit >20 mg/l (Neiman, *et al.*, 1949)[86], and for all procedures interference from other nitroaromatics would be expected.

Three papers describe the gravimetric determination of picric acid by precipitation with Nitron, which is alleged to be 2,5,6-triphenyl-2,3,5,6-tetraazabicyclo[2:1:1]hex-3-ene (Busch and Blume, 1908; Cope and Barab, 1917; Baker, 1954)[91,92,93]. However, since the solubility of nitron picrate in water exceeds 1 mg/l (Baker, 1954)[93], this procedure is of limited use for wastewaters, as is the gravimetric method of Pasini and Vercellone (1954)[94], which employs 4-octoxyphenylguanidinium chloride.

The single reported procedure for trace analysis of picramic acid in water involves extraction with a cationic dye into an organic solvent, followed by photometric determination (Koreman, *et al.*, 1968)[79]. Sensitivities of 0.5-1 mg/l are claimed. Procedures have been described for isolation of picramic acid by paper chromatography (Gemzova and Gasparic, 1966)[95] and tlc (Parihar, *et al.*, 1966)[81].

Analysis of tetryl in soil samples by glc using flame-ionization detection has been described (Small and Rosenblatt, 1974)[4]. This analysis was performed using a 6 foot column containing 3% OV-1 on 100/200 mesh Chromosorb W at 220°C and helium as a carrier gas.

Discussion. No data was recovered concerning the toxicity of tetryl to aquatic organisms. However, in natural waters tetryl is slowly degraded to picric acid, for which considerable data exists. For fish, the minimum toxic level reported is 30 mg/l, while for microorganisms the tolerance varies from a few ppm for some amoebae to >3000 ppm for some bacteria. Two considerations qualify any interpretation of available toxicity data, particularly in the older literature. First, picric acid is nearly completely dissociated in water; the toxic effects reported in some cases may have been due in part to a low pH. Second, 2,4-dinitrophenol, which has been shown to be much more toxic for many organisms, may be a common contaminant of commercial grade picric acid.

Although substantial data are lacking, picramic acid, an important metabolite of picric acid, does not appear to be much more toxic than picric acid. At this time we have recovered no information indicating that wastewater effluents containing picric acid or picramic acid in any anticipated concentration would present an environmental hazard, either directly or indirectly.

Recommendations for Further Work.

1. The environmental chemistry of aqueous tetryl should be studied to establish the rate of decomposition under natural conditions of temperature, pH and sunlight. The rates and products of microbial degradation of tetryl, picric acid and picramic acid should be examined. The intent of these studies is to determine the rate of disappearance of these compounds from receiving waters.

2. Acute, 96-hour median tolerance levels of tetryl, picric acid and picramic acid should be established for appropriate freshwater and marine target species, such as the rainbow trout (*Salmo gairdneri*), common killifish (*Fundulus heteroclitus*) and *Daphnia*. Chronic toxicity studies should be carried out over the life cycle of organisms used in acute studies. The effects of environmental variables on toxicity should be included, in particular the effect of pH on picric and picramic acids.

TRINITRORESORCINOL AND LEAD STYPHNATE

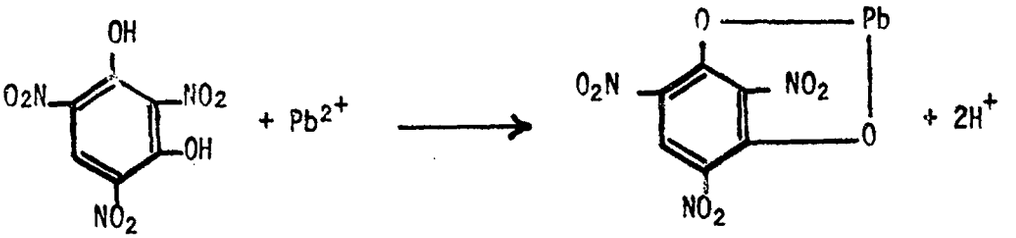
Approach to Toxicity to Aquatic Organisms. A survey of the literature through July 1974 revealed no data concerning the toxicity of lead styphnate or styphnic acid (trinitroresorcinol) to aquatic organisms. The survey scheme for *Chemical Abstracts* is presented in Table 8. For Volumes 1-78 (1907-1973), the appropriate index headings were surveyed; for Volumes 79-80, the keyword citations were surveyed issue by issue. Secondary sources, primarily studies on mammalian toxicology, provided no aquatic references.

Aquatic Chemistry of Trinitroresorcinol and Lead Styphnate. Trinitroresorcinol reacts with lead (II) salts to form a number of different

Table 8. Literature Survey Scheme for Lead Styphnate and Styphnic Acid

Citation	Chemical Abstracts Vol. 80	79	78	77	76	75-66	65-56	55-51	50-1
Lead Styphnate	X	X						X	X
Styphnic Acid, Lead complex	X	X						X	X
2,4-Dioxa-3-plumbabicyclo-[3.3.1]-nona-1(9),5,7-triene, 3,3-didehydro-6,8,9-trinitro-	X	X	X	X	X	X	X		
Resorcinol, 2,4,6-trinitro-	X	X		X	X	X	X	X	X
Trinitroresorcinol	X	X							
1,3-Benzenediol, 2,4,6-trinitro-	X	X	X						

products, of which the most stable by far is the 1:1 complex (Durtschi, *et al.*, 1969)[96]. This material is generally assigned the bridged bicyclic structure below, although other structures have been proposed (Zingaro, 1954)[97].



The chemistry of lead styphnate has been briefly reviewed by Small and Rosenblatt (1974)[4], who note that its solubility in cold water is reported to be 400 mg/l, compared with 6.8 mg/l for trinitroresorcinol at 25°C. It is not known whether dissolved lead styphnate is completely dissociated or partly complexed.

Analysis of Trinitroresorcinol and Lead Styphnate. No completely satisfactory procedure for trace estimation of trinitroresorcinol in wastewater has been devised. A Russian paper claims a detection limit of *ca.* 0.1 mg/l for an extraction-photometric procedure using cationic dyes as complexing agents (Korenman, *et al.*, 1968)[98]. In a method recently described (Norwitz and Gordon, 1974)[99], a sample containing trinitroresorcinol is acidified with perchloric acid and extracted several times with methylene chloride. The absorbance at 273 nm is compared with standards, giving an apparent detection limit of 0.5-1.0 mg/l. Separation of mixtures containing trinitroresorcinol and identification has been achieved using paper chromatography (Colman, 1963; Gasparič, 1964)[100,101] and tlc (Parihar, *et al.*, 1964)[81].

There is no general procedure for estimation of lead styphnate in water, and considering that lead styphnate is not exceptionally stable in water, it may not be practical to devise one. Identification of lead styphnate by tlc has been described, but this procedure is apparently designed for analysis of explosive mixtures (Boehm, 1968)[102].

Recommendations for Further Work.

1. The toxicity of trinitroresorcinol to a variety of aquatic organisms should be examined using appropriate target species. Acute 96-hour median tolerance limits should be determined for typical freshwater and marine species such as the bluegill (*Lepomis macrocirus*), common killifish (*Fundulus heteroclitus*) and *Daphnia*, and chronic toxicities should be investigated over the life cycle of the same organisms. The toxicity of trinitroresorcinol to common wastewater microorganisms should also be studied.

The acute toxicity of lead styphnate for the same organisms should be examined, and compared with the toxicities of soluble lead (II) salts. Assuming that lead styphnate will prove to be more toxic than trinitroresorcinol, this would establish whether lead styphnate is uniquely toxic or owes its toxicity solely to free lead (II) ions.

2. The rate and products of biodegradation of trinitroresorcinol should be determined. If a stable intermediate product is formed (such as one with a nitro group reduced to an amino group), it would be advisable to examine the toxicity of this material as well.

The photochemistry of aqueous trinitroresorcinol and lead styphnate should be studied to ascertain whether primer wastewaters exposed to sunlight produce photolysis products, and whether these products are more toxic.

PENTAERYTHRITOL TETRANITRATE (PETN)

Approach to Toxicity to Aquatic Organisms. A survey of the literature through July 1974, revealed no data concerning the toxicity of PETN to aquatic organisms of any kind. The survey scheme for *Chemical Abstracts* is presented in Table 9. For Volumes 1-78 (1907-1973) the appropriate index headings were surveyed; for Volumes 79-80 (1973-1974) the keyword citations were surveyed issue by issue.

Table 9. Literature Survey Scheme for PETN

Citation	Chemical Abstracts Vol. 80	79	78	77	76	75-66	65-56	55-51	50-1
Pentaerythritol tetranitrate	X	X				X	X	X	X
PETN	X	X				X	X	X	X
Propanediol, 2,2-bis(nitrooxy) methyl, dinitrate	X	X	X	X	X				

Secondary sources, primarily studies on mammalian toxicology and pharmacodynamics, provided no aquatic references, and a computer search by J. L. Mullaney was also negative. Medline (January 1964 to June 1973), Toxline (1940-1973), NTIS (1964 to July 1963) and *Chemical Abstracts Condensates* (July 1970 to July 1973) were accessed for pentaerythritol tetranitrate.

Biotransformations of PETN. In the section of the chemistry of pentaerythritol tetranitrate it is shown that PETN is capable of stepwise hydrolysis to pentaerythritol and nitrate ion. Although this transformation can in principle occur in living organisms, the hydrolysis

reaction does not appear to be very significant in mammalian systems. Studies both *in vitro* and *in vivo* support the conclusion that nitrite rather than nitrate is the product of biodegradation of PETN as well as other nitrate esters (Ikawa, *et al.*, 1959; Needleman and Hunter, 1965; Di Carlo, *et al.*, 1967)[103,104,105].

Extensive investigations at the Warner-Lambert Research Institute and elsewhere have shown that PETN is readily degraded to pentaerythritol trinitrate and nitrite ion *in vitro* by rat liver homogenase (Ikawa, *et al.*, 1959)[103], by rat liver nitrate reductase (Needleman and Hunter, 1965)[104], by human whole blood, plasma and red cell suspension (Di Carlo, *et al.*, 1965)[106], by rat blood plasma and erythrocytes (Di Carlo, *et al.*, 1965)[107], by both mouse liver parenchymal and reticuloendothelial cells (Di Carlo, *et al.*, 1967)[105], and by all rat heart subcellular fractions (Di Carlo, *et al.*, 1967)[108]. Studies *in vivo* indicate that PETN is biotransformed not only by blood, but even more effectively by heart, liver and spleen (Di Carlo, *et al.*, 1967; Di Carlo, 1967)[109,110]. Adsorption of PETN administered to rats was found to be much faster from the large intestine than from the stomach (Di Carlo, *et al.*, 1967)[111]. This difference in absorption has been attributed to degradation of PETN to the mono-, di-, and tri-nitrates by the bacterial flora of the intestine, and the more ready absorption of the metabolites. The literature on organic nitrate metabolism published before 1971 has been reviewed by Litchfield (1971)[112].

Earlier relative rate studies indicated that the metabolism of PETN by rat blood occurs stepwise, with denitration becoming progressively slower with each step (Di Carlo, *et al.*, 1965)[107]. Needleman and Hunter (1965)[104], on the other hand, found that the trinitrate was denitrated 6-7 times faster than PETN by soluble liver nitrate reductase. The differences observed may be due to the fact that whole erythrocytes, principally responsible for denitration in whole blood, bind PETN much more firmly than trinitrate. However, insofar as PETN is absorbed from the digestive tract as one or more of its metabolites, the rate of degradation of PETN itself by the blood or organs is not relevant to the mechanism of biotransformation of the drug. For this reason, recent studies by the Warner-Lambert and Wake Forest groups have been concerned with the biotransformation of pentaerythritol trinitrate (Di Carlo, *et al.*, 1969; Di Carlo, *et al.*, 1969; Davidson, *et al.*, 1971; Crew, *et al.*, 1971; Leinweber, *et al.*, 1974; Melgar, *et al.*, 1974)[113,114,115,116,117,118]. This ester is rapidly absorbed following oral administration, and is degraded stepwise to the dinitrate and mononitrate *in vivo*. Of

particular interest is the discovery of the glucuronic and conjugates of pentaerythritol mono-, di- and tri-nitrates in the biliary excretion of rats to which ^{14}C-pentaerythritol trinitrate has been administered[116,117]. The mononitrate and dinitrate glucuronides appear in the urine along with the free mononitrate, pentaerythritol, and traces of the trinitrate glucuronide. The investigators have presented evidence for the equilibria described in Figure 2, and suggest that gastrointestinal resorption and recirculation of the free nitrate esters may be important (Melgar, *et al.*, 1974)[118].

The reductive degradation of PETN and the free trinitrate, dinitrate, and mononitrate esters has been shown to be effected by glutathione (GSH), catalyzed by cytosol, in the following sequence of reactions[105]:

$$2\ \text{Glucose-6-PO}_4 + 2\text{NADP} \xrightarrow{\text{dehydrogenase}} 2\ \text{gluconolactone-6-PO}_4 + 2\text{NADPH}$$

$$2\ \text{NADPH} + \text{GSSG} \xrightarrow{\text{reductase}} 2\text{NADP} + 2\text{GSH}$$

$$2\text{GSH} + \text{PETN} \xrightarrow{\text{reductase}} \text{GSSG} + \text{PE-trinitrate nitrite} + \text{H}_2\text{O}$$

$$\text{PE-trinitrate nitrite} + \text{H}_2\text{O} \xrightarrow{\text{hydrolase}} \text{PE-trinitrate} + \text{HNO}_2$$

For degradation of the nitrate ester glucuronides, NADPH is more effective than glutathione[118], i.e.,

$$\text{PE-trinitrate glucuronide} + \text{NADPH} \xrightarrow[\text{hydrolase}]{\text{reductase}} \text{PE-dinitrate glucuronide} + \text{HNO}_2$$

Of the PETN metabolites, the trinitrate is most susceptible to further denitration. The dinitrate is less reactive, and the mononitrate is degraded only slightly and apparently nonenzymatically. The glucuronyl group greatly inhibits denitration: the trinitrate glucuronide is only as susceptible as the free dinitrate, the dinitrate glucuronide is

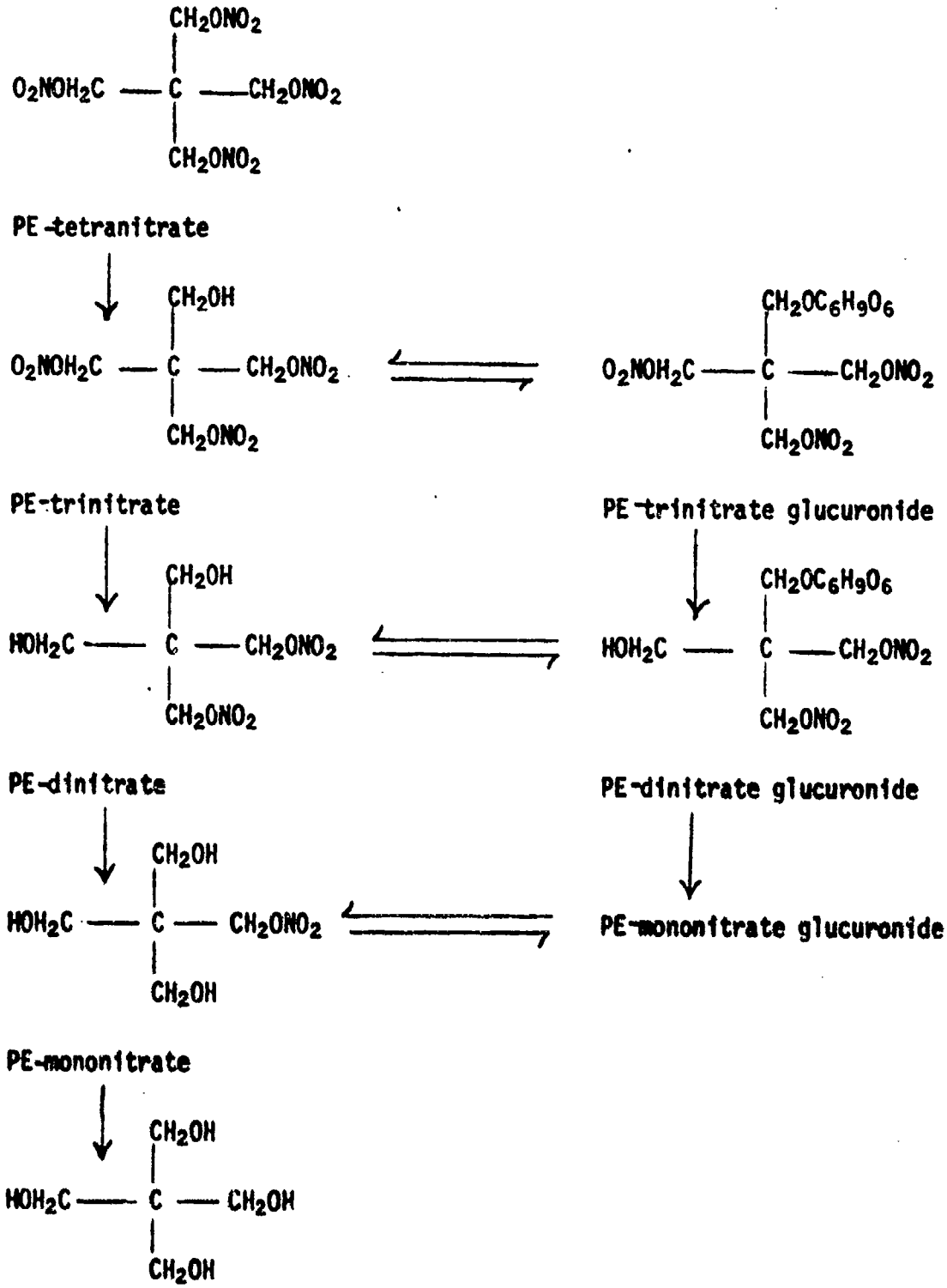


Figure 2. Metabolism of PETN in the Rat (Me gar, *et al.*, 1974)[118]

degraded very slowly, and the mononitrate glucuronide is unreactive. Formation of the glucuronides has been effected *in vitro* using uridine diphosphate glucuronic acid catalyzed by liver microsomal protein. In the case of the mono- and di-nitrates, apparently only the monoglucuronide is formed. The reverse reaction is brought about by lysosome glucuronidase.

The present view is that PETN metabolites are subject to enterohepatic recirculation, i.e., the metabolite is converted into its glucuronide in the liver and excreted via the bile into the intestine, where it is deconjugated by the intestinal bacteria and resorbed into the blood stream as the free nitrate[118]. A current paper reviews the status of nitrate ester metabolism, including hydrolysis (Litchfield, 1974)[119].

Chemistry of PETN. The chemical properties of pentaerythritol tetranitrate have been reviewed by Small and Rosenblatt (1974)[4]. This ester would be expected to undergo sequential hydrolysis in water, as shown in Figure 3. Aubertein and Rehling (1959)[120] investigated the rate of hydrolysis of PETN in hot water under different conditions of temperature and pH, but did not identify intermediate products. In the range 90-120°C, the overall rate is strongly temperature dependent and is strongly accelerated by acid or base. The water solubility of PETN is so low (*ca.* 2 mg/l at 20°C) that almost all hydrolysis studies have been carried out in mixed solvents, namely 90% ethanol (Schulek, *et al.*, 1960; Fraser, 1968; and Fraser, 1968)[121,122,123] and 75% aqueous dioxane (Di Carlo, *et al.*, 1964)[124] at reflux temperatures. Furthermore, the reactions studied were promoted by strong acid or strong base. Thus, the experimental data are not directly applicable to the assessment of hydrolysis of PETN in natural waters and wastewaters. Di Carlo, *et al.* (1964)[124] have studied the sequential hydrolysis of ^{14}C-labeled PETN in aqueous dioxane, promoted by 1N-hydrochloric acid. Rate data are summarized in Table 10, and it is seen that all four denitration steps proceed at a moderate rate.

Table 10. Recovery of ^{14}C in Hydrolysis Mixtures of ^{14}C-Labelled PETN (Di Carlo, *et al.*, 1964)[124]

Compound	% of ^{14}C Identified after		
	4 hrs	5 hrs	6 hrs
PETN	39	15.0	5.6
PE-trinitrate	39	28.0	11.0
PE-dinitrate	23	35.0	28.0
PE-mononitrate	0	13.0	26.0
Pentaerythritol	0	7.7	32.0

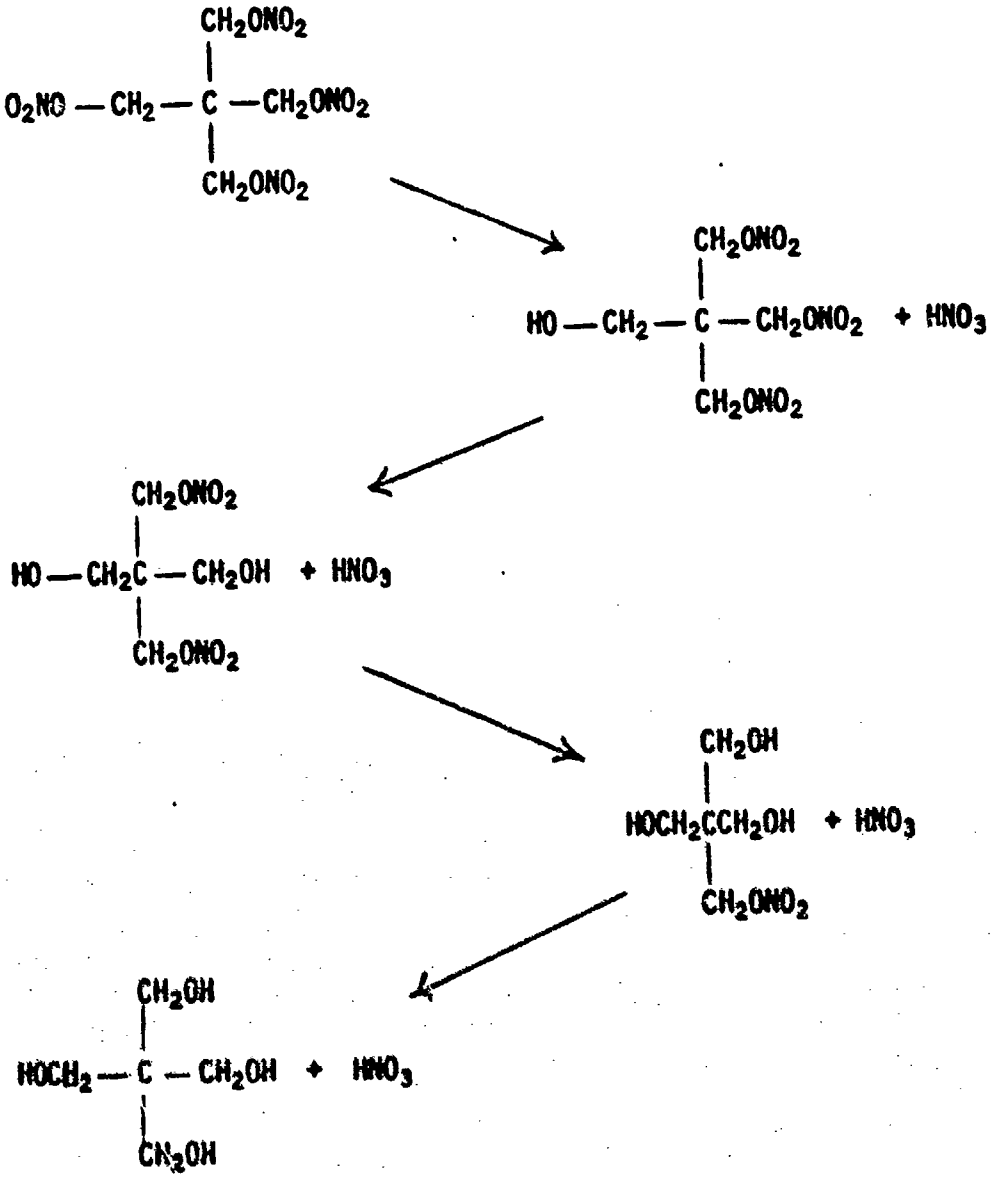


Figure 3. Hydrolysis of PETN

According to Schulek, *et al.* (1960)[121], the base-promoted denitration proceeds with many side reactions. Almost as much nitrite is produced from the reaction as nitrate, and a small quantity of ammonia is also produced. The authors did not establish whether the reduction products were formed in the saponification steps or in subsequent steps, but it is not unreasonable to suppose that the reducing agent is formaldehyde, formed in the retroaldol reaction of pentaerythritol.

Two papers report on the decompositon of solid PETN exposed to u.v. light (Betti and Merli, 1941; Urbanski and Malendowicz, 1938)[125,126], but it is not known whether this relates in any way to degradation of dissolved PETN and its metabolites in the environment.

Analysis of PETN. There is no completely satisfactory procedure for estimation of PETN and its metabolites in water. When investigating the hydrolysis and later, the metabolism of ^{14}C-labeled PETN, Di Carlo, *et al.* (1964)[124] devised a scheme for separation of the metabolites by thin-layer chromatography and estimation by radioscanning. This procedure is highly accurate, but it is only applicable to certain research problems.

In a procedure described by Davidson, *et al.* (1971)[127], PETN and its metabolites are treated in chloroform or methylene chloride with trifluoroacetic anhydride, thereby trifluoroacetylating the free hydroxyl groups of the metabolites. Using gas chromatography with electron capture detection, the individual esters are identified and estimated at levels of 0.25 to 2 ng, depending on the number of trifluoroacetyl groups. This procedure is of limited use for wastewater samples because of incomplete extraction of the metabolites, which can be expected to be progressively less soluble in nonpolar solvents, such as methylene chloride, and more soluble in water with an increasing degree of denitration. It is not certain, e.g., that pentaerythritol could be extracted from water at all.

Norwitz and Gordon (1973)[128] have reported a colorimetric procedure for estimation of PETN in wastewater. The nitrate ester is extracted with methylene chloride, and the residue after evaporation of the solvent is treated with sulfuric acid. The nitric acid released is then estimated by means of its reaction with phenoldisulfonic acid (American Public Health Assn., 1971)[129]. The reported precision is about 0.1 mg/l,

and neither inorganic nitrates nor aromatic nitro compounds interfere. However, any nitrate ester extracted into methylene chloride will be detected as PETN. Golubeva (1952)[130] has claimed a sensitivity of 0.5 mg/l for the same general method. A similar procedure was earlier described by Yagoda (1943)[131], who extracted nitrate esters from biological fluids with ether. The nitric acid produced by the action of sulfuric acid on the residue was allowed to react with m-xylenol. The colored nitroxylenol was distilled directly into a Nessler tube for estimation. The precision and limitations of this procedure are about the same as for the method of Norwitz and Gordon[128]. It should be noted that the precision for these methods can usually be improved substantially by continuous extraction of larger samples.

Most procedures for PETN have been developed for analysis of drugs or explosives. Components of mixtures have been identified by paper chromatography (Krien, 1963)[17] and tlc (Prat and Forestier, 1963; Bohm, 1966; and Bohm, 1967; Hennig, 1966; Barnes, 1967)[20,19,132,133,134]. Parihar, *et al.* (1967)[135] have adapted tlc to quantitative determination of various explosive nitrates. They claim that 2-4 µg are conveniently estimated. For a similar procedure, Hansson (1963)[77] reports a detection limit of about 1 µg. A qualitative procedure based on i.r. spectroscopy has also been reported (Carol, 1960)[136].

Recommendations for Further Work.

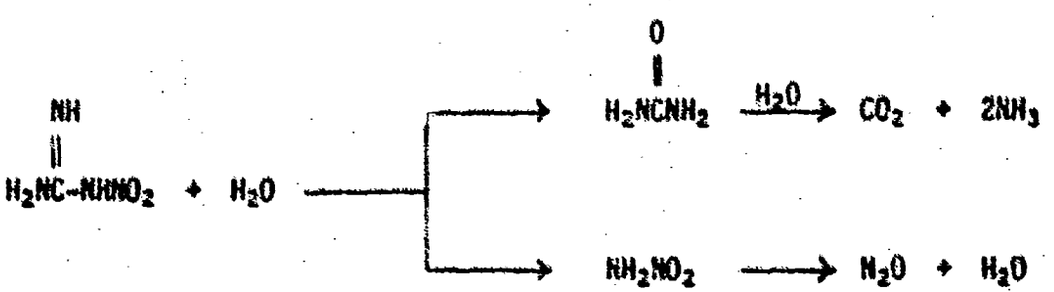
1. The environmental chemistry of aquatic pentaerythritol tetranitrate, trinitrate, dinitrate and mononitrate (hereafter PETN and its metabolites) should be studied to establish the rates of denitration of each ester under natural conditions of temperature, pH and sunlight. The rates and products of microbial degradation of PETN and its metabolites should be examined. The intent of these studies should be to determine the rate of disappearance of PETN and its metabolites from receiving waters.

2. Acute, 96-hour median tolerance limits of PETN and its metabolites should be established for appropriate freshwater and marine target species, such as rainbow trout (*Salmo gairdnerii*), common killifish (*Fundulus heteroclitus*) and *Daphnia*. Quite probably, 96-hour TLm's will exceed the water solubility of PETN and some of its metabolites, and it will be necessary to work with suspensions of these materials. Chronic toxicity studies should be carried out over the life cycle of organisms used in acute studies. To assure that any observed injurious effects are biochemical and not physical, concentrations of PETN and its metabolites should not exceed the solubility limit.

NITROGUANIDINE

Approach to Toxicity to Aquatic Organisms. A survey of the literature through July 1974 revealed no data concerning the toxicity of nitroguanidine to aquatic organisms. All references to nitroguanidine (or guanidine, nitro) listed in *Chemical Abstracts* were surveyed. For Volumes 1-78 (1907-1973) appropriate index headings were surveyed, while for Volumes 79-80 (1973-1974) the keyword citations were scanned. Secondary sources, primarily studies on mammalian toxicology, provided no aquatic references.

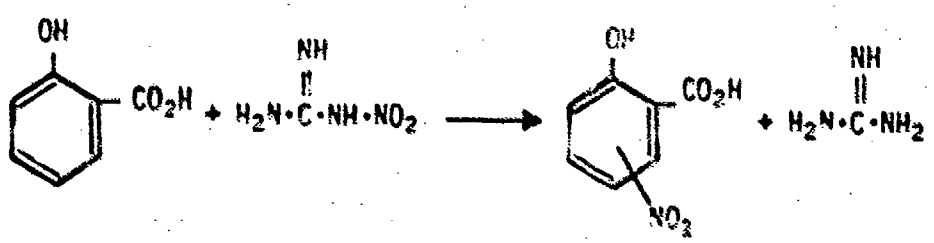
Aquatic Chemistry. The chemistry of nitroguanidine and its manufacture have been reviewed by Small and Rosenblatt (1974)[4], who note that the solubility of nitroguanidine in water is 4.4 g/l at 25°C, far greater than most common explosives. Nitroguanidine is known to undergo hydrolytic degradation under strongly acidic (Davis, 1922)[137] or alkaline (Fry and Treon, 1925)[138] conditions to yield ammonia, carbon dioxide, and nitrous oxide, probably through nitramide and urea.



It is not reported whether this reaction is rapid enough under natural water conditions to introduce significant quantities of nitrous oxide into the environment. Nitroguanidine hydrolysis has been reviewed by McKay (1952)[139].

Analysis of Nitroguanidine. There is no satisfactory procedure for trace analysis of nitroguanidine in waters and wastewaters. Most of the methods for estimation of nitroguanidine involve chemical reduction of the nitro group with a standard reagent, such as titanium (III) (Kouba, *et al.*, 1948; Brand, *et al.*, 1955; Roth and Wegman, 1958)[140,141,142] or chromium

(III) (Botei and Furman, 1955)[143] chloride. These reagents are not specific for nitroguanidine; other organic nitro compounds are readily reduced. In the procedure of Roth and Wegman (1958)[142] explosive mixtures are analyzed for nitroguanidine by mixing with water and extracting with an organic solvent such as carbon tetrachloride, in which most organic nitro compounds except nitroguanidine are readily soluble. The aqueous portion is buffered and treated with titanous chloride, and the excess reducing agent is back-titrated with ferric alum. The results of Roth and Wegman suggest that the procedure could be adapted for wastewater analysis with a detection limit of a few mg/l. Because the extent of reduction of nitroguanidine may vary unless conditions are precisely controlled, Stalcup and Williams (1955)[144] have devised a procedure involving transnitration of salicylic acid with nitroguanidine. The product is cleanly reduced by titanium (III) chloride. The various methods for determination of nitroguanidine



nitrogen have been evaluated by Fauth and Stalcup (1958)[145]. Polarographic methods have been reported (Atkain, *et al.*, 1966; Opel and Ardelt, 1967)[146,147], and would probably give about the same detection limit.

Two other methods might be suitable for analysis of nitroguanidine in greater than trace concentrations. Nomura, *et al.* (1969)[148] have devised a procedure for analysis of urea type compounds by liquid chromatography in aqueous acid solution. The detection limit for nitroguanidine is not stated, but apparently exceeds 10 mg/l. A spectrophotometric method described by DeVries and Gants (1953)[149] is capable of detecting nitroguanidine at the mg/l level by means of its adsorption at 265 nm. For wastewater, this procedure would be subject to interference by many substances.

Recommendations for Further Work.

1. The environmental chemistry of nitroguanidine should be studied to establish the rates and products of conversion effected in water under natural conditions of temperature, pH and sunlight. The rates and products of microbial degradation of nitroguanidine should be examined. The intent of these studies is to determine the rate of disappearance of nitroguanidine from receiving waters. In this regard, there is a pressing need for an analytical procedure capable of detecting nitroguanidine at a level not exceeding 0.1 mg/l (10^{-6}M) in wastewater and biological fluids.

2. The acute, 96-hour median tolerance limits of nitroguanidine and any important degradation products should be measured for appropriate freshwater and marine target species, such as bluegill (*Lepomis macrochirus*), common killifish (*Fundulus heteroclitus*) and *Daphnia*. Chronic toxicity studies should be carried out over the life cycle of the same organisms.

TETRACENE

Approach to Toxicity to Aquatic Organisms. A survey of the literature through July 1974 revealed no data concerning the toxicity of tetracene to aquatic organisms of any kind. The survey scheme for *Chemical Abstracts* is presented in Table 11. For Volumes 1-78 (1907-1973), the appropriate index headings were surveyed; for Volumes 79-80 (1973-1974), the keyword citations were surveyed issue by issue. Because three different structures have been proposed, it has been necessary to survey all three.

Chemistry of Tetracene. Tetracene or tetrazene has been variously assigned three different structures (Patinkin, *et al.*, 1955; Duke, 1971)[150 151], of which the most recent and probably best supported is:

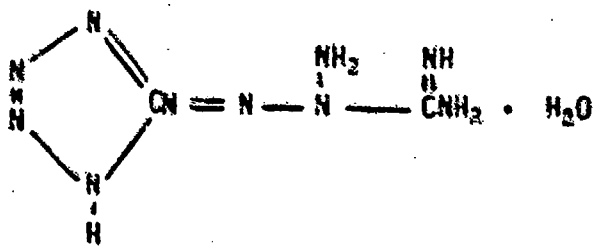
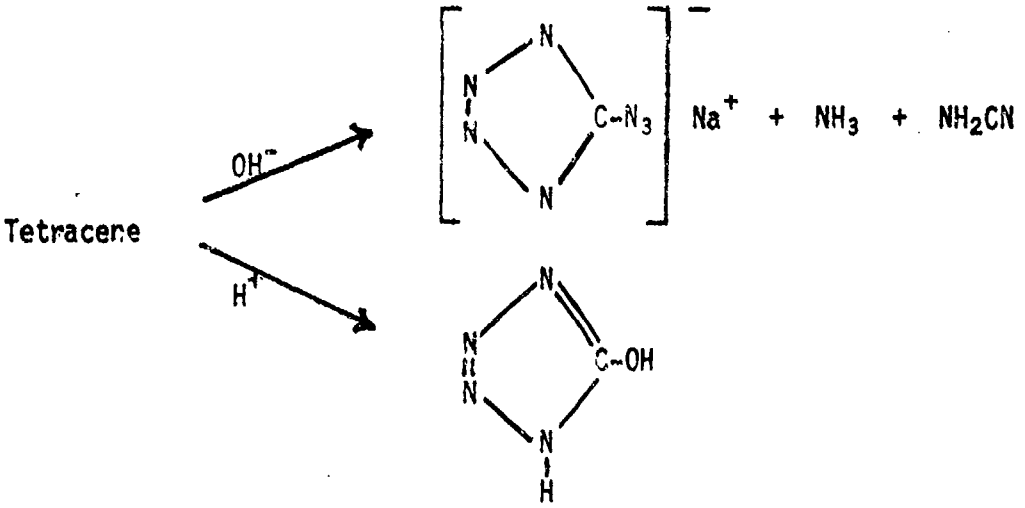


Table 11. Literature Survey for Tetracene

Citation	Chemical Abstracts Vol. 80	79	78	77	76	75-66	65-56	55-51	50-41	40-1
Tetracene (explosive)	X	X					X	X	X	X
Tetrazene (explosive)	X	X				X	X	X	X	X
1-Tetrazene, 4-guanyl-1-(nitrosoaminoguanyl)	X	X								X
1-Tetrazene, 4-amidino-1-(nitrosaminoamidino)-	X	X		X	X	X	X	X	X	
1-Tetrazene-2-carboximidamide, 4-(1H-tetrazol-5-yl)-, monohydrate	X	X	X	X	X					
3-Tetrazene-1-carboxamidine, 4-(1H-tetrazol-5-yl), monohydrate	X	X		X	X	X				

The chemistry of tetracene has been reviewed by Patinkin, *et al.* (1955)[150] and briefly by Small and Rosenblatt (1974)[4] who note that it is considered to be insoluble in water. This may reflect difficulty in dissolving the pure crystals rather than true insolubility, however, since the material is known to be readily soluble in dilute acid. The decomposition of tetracene in alkali was found to produce ammonia, cyanamide and the sodium salt of 5-azidotetrazol, while in dilute acid tetracene yields 5-hydroxytetrazol.



Analysis of Tetracene. There is not procedure reported for trace analysis of tetracene in water. Wild (1957)[152] has described a polarographic method suitable for estimating tetracene in the concentration range 20-200 mg/l. Interference in the analysis from lead styphnate has been discussed by Traas and Ligtenberg (1962)[153] and by Wild (1963)[154]. A possible chemical basis for trace analysis of tetracene in water is provided by the reaction with β-naphthol to produce an azo dye (shreve, *et al.*, 1974; Reilly, *et al.*, 1948)[155 156]. It may be profitable to seek a spectrophotometric procedure for estimation of tetracene based on this reaction.

Recommendations for Further Work.

1. The environmental chemistry of tetracene should be studied to establish the rates and products of conversion effected in water under natural conditions of temperature, pH and sunlight. The rates and products of microbial degradation of tetracene should be examined. The intent of these studies is to determine the rate of disappearance of tetracene from receiving waters. However, it will first be necessary to develop an analytical procedure capable of detecting tetracene at a level not exceeding 1 mg/l in wastewaters.

2. The acute, 96-hour median tolerance limit of tetracene and any important degradation products should be measured for appropriate freshwater and marine target species, such as bluegill (*Lepomis macrochirus*), common killifish (*Fundulus heteroclitus*) and *Daphnia*. Chronic toxicity studies should be carried out over the life cycle of the same organisms.

RED PHOSPHORUS

Approach to Toxicity to Aquatic Organisms. In the course of preparation of a report on the mammalian and aquatic toxicities of white phosphorus (also called yellow phosphorus), it was attempted to recover all references to elemental phosphorus (Burrows and Dacre, 1974)[157]. No evidence was revealed that red phosphorus is toxic to any organism, regardless of the concentration or mode of administration. To update the earlier literature study, all phosphorus citations in Volumes 79-80 (1973-1974) of *Chemical Abstracts* were surveyed, issue by issue. Again no reference to red phosphorus toxicity was noted. The literature review of the aquatic toxicity of phosphine has been updated by surveying Volumes 79-80 of *Chemical Abstracts*. No additional information was recovered.

Discussion. Red phosphorus has a much lower volatility and presumably much lower water solubility than the white form. Van Wazer (1958)[158] has noted that commercial red phosphorus is a moderately unstable material, reacting slowly with water vapor to form phosphine and a mixture of oxyacids of phosphorus. As noted in the earlier report, there is some evidence that phosphine is toxic to fish, but it is probably readily biodegradable. The oxyacids should present no environmental hazard.

In the absence of definitive toxicity data for phosphine, it cannot be stated with certainity that red phosphorus is completely harmless; quite possible a heavy accumulation of red phosphorus could present a localized pollution problem. Furthermore, it is possible that a high concentration of suspended red phosphorus particles could be injurious to the gills of fishes. However, Small and Rosenblatt (1974)[4] have presented evidence that the maximum discharge of red phosphorus from tracer shall loading facilities at Lake City Army Ammunition Plant would be 0.7 lb/day. Hence, considering the largest concentrations of red phosphorus likely to be encountered, the potential hazard does not justify further study of the aquatic toxicity. This conclusion could be altered if studies recommended in the earlier report showed phosphine to be unusually toxic and biorefractory.

POTASSIUM PERCHLORATE

Approach to Toxicity to Aquatic Organisms. The published literature has been surveyed through July, 1974, by means of *Chemical Abstracts*, using the survey scheme presented in Table 12. For Volumes 1-78 (1907-1973), the appropriate index headings were surveyed; for Volumes 79-80 (1973-1974), the keyword citations were surveyed issue by issue. Because it seems certain that perchlorate ion alone is responsible for the biological effects of potassium perchlorate, the literature relating to all simple, soluble perchlorates was examined. A few additional relevant papers were disclosed in the bibliographies of the primary references. A computer search by J. L. Mullaney provided no additional pertinent references. *Medline* (July 1969 to June 1973), *Toxline* (1940 to 1973), *NTIS* (1964 to July 1973) and *Chemical Abstracts Condensates* (July 1970 to July 1973) were accessed for *potassium perchlorate*.

Table 12. Literature Survey Scheme for Potassium Perchlorate

Citation	Chemical Abstracts Vol. 80	79	78	77	76	75-65	65-56	55-51	50-41	40-1
Perchlorate	X	X	X	X	X	X	X	X	X	X
Perchloric acid	X	X	X	X	X	X	X	X	X	X
potassium salt			X	X	X	X				
sodium salt			X	X	X	X				
Alkali metal perchlorate	X	X					X	X	X	X
Potassium perchlorate	X	X					X	X	X	X
Sodium perchlorate	X	X					X	X	X	X

Toxicity of Perchlorate to Aquatic Organisms.

Fish. The literature of acute perchlorate toxicity to teleost fishes appears to comprise a single paper by Kahane (1936)[159], who found that goldfish exhibit no evidence of distress at sodium perchlorate levels below 1000 mg/l (800 mg/l as ClO_4) (Table 13). At levels exceeding 2000 mg/l, symptoms of asphyxiation are noticeable, and mortality is complete in a few hours at 20,000 mg/l. This represents an order of acute toxicity so low that perchlorate can probably be dismissed as an environmental hazard to freshwater fishes, although a few corroborative studies with more sensitive fish, such as the rainbow trout, would probably be desirable, as would similar studies with at least one marine fish.

The chronic effect of perchlorate on fish has been studied by Pflugfelder (1959)[160], who exposed *Lebistes reticulatus* (guppies) to 500 mg/l of potassium perchlorate (360 mg/l as ClO_4) for a year and longer. Gross enlargement of the thyroid was followed by inactivation of the thyroid, and sexual development was arrested. Insofar as the toxic action of perchlorate is antithyroidal, the response could vary widely among fishes. The goldfish (*Carassius auratus*) has been shown to have a very inactive thyroid compared with *Lebistes reticulatus*

Table 13. Toxicity of Sodium Perchlorate to Goldfish (Kahane, 1936)[159]

ClO_4 Concn. mg/l	No. of Fish	No. Surviving 10 hr.	No. Surviving 24 hr.	No. Surviving 72 hr.
160	5	5	5	5
320	5	5	5	5
800	5	5	5	5
1600	5	5	4	
4000	5	5	3	
8000	5	5	3	
16000	3	0		
32000	3	0		

(Fortune, 1956)[161], and would therefore be expected to exhibit greater tolerance to perchlorate. Fairly high perchlorate levels can inhibit development of eggs and embryos of various aquatic animals and modify the metamorphosis of sea lamprey larvae (*vide infra*). It is not unlikely that some moderate level of perchlorate ion will have an adverse effect on the development of the eggs of teleost fishes. This merits investigation.

The only other reference to the effect of perchlorate on fishes is contained in a paper by Sterba and Schneider (1961)[162], who found that metamorphosis of 2-4 year old ammocoetes (larvae) of the sea lamprey, *Lampetra planeri*, is not hindered by 9 months exposure to 500 mg/l of potassium perchlorate (360 mg/l as ClO_4). There is, however, some tendency to premature metamorphosis of the fins of the exposed larvae, and the eyes show anomalous development. The authors reported 40% mortality in the first eight weeks for all larvae exposed to perchlorate, compared with 30% for the controls, a difference which is not statistically compelling.

Amphibia. Newts, salamanders, frogs and toads have all been the subjects of study of the physiological action of perchlorate. Dent and Lynn (1959)[163] have conducted comparative studies on the effect of perchlorate injected into the body cavity of American newt, *Triturus*

(Diemyctylus) viridescens and a terrestrial counterpart, the dusky salamander, *Desmognathus fuscus*. Injection of 0.1 ml of 0.2% aqueous potassium perchlorate (approx. 100 mg/kg) on alternate days for 30 days produced significant histological changes in the salamander but not the newt. The effect of perchlorate is to prevent accumulation of iodine by the thyroid, thereby blocking synthesis of thyroid hormone, which results in increased release of thyroid stimulating hormone (TSH) by the pituitary. TSH, in turn, brings about the histological changes in the thyroid. Apparently the normal inactivity of the thyroid of *Triturus* is due to the low production of TSH by the pituitary. Since thyroid activity is commonly sensitive to temperature, Dent and Lynn suggest that the inactive thyroid of *Triturus* is related to the wide range of environmental temperatures within which it thrives. *Desmognathus*, in contrast, lives under more restricted temperature conditions. Fortune (1956)[161] has used this argument to rationalize the differences in thyroid activity of goldfish and other teleost fishes. Dent (1961)[164] has found that continuous immersion of *Triturus* in water containing 50 mg/l of potassium perchlorate (36 mg/l as ClO_4) for 180 to 450 days resulted in significant histological changes in the thyroid and pituitary. The thyroid was involuted (atrophied) and large cells appeared in the pituitary characteristic of animals from which the thyroid has been surgically removed. Overt changes included thickening of the epithelia and increased superficial pigmentation, apparently related to the fact that thyroid hormone normally brings about the shedding of the skin in newts. Dent reported 20% mortality for all exposed animals without specifying the period of exposure.

Pflugfelder (1959)[160] found that metamorphosis of the tadpoles of the clawed toad, *Xenopus laevis*, as well as a frog, *Rana temporaria*, is completely inhibited by exposure to 500 mg/l potassium perchlorate (360 mg/l as ClO_4) shortly after hatching. The tadpoles continue to grow beyond normal size, and the inhibition of metamorphosis may become irreversible in time. Pflugfelder and Schubert (1965)[165] have reported on changes occurring in the skin of *Xenopus laevis* exposed and unexposed to potassium perchlorate during metamorphosis. Inhibition of thyroid action has been implicated by Hourdry (1973)[166] in a study of the effect of perchlorate on tadpoles of a toad, *Discoglossus pictus*. In the normal tadpole the intestine, a large spiral, diminishes in volume and loses its spiral nature during metamorphosis. Tadpoles maintained in solutions containing 1000 mg/l of potassium perchlorate (720 mg/l as ClO_4) for 4-35 days do not exhibit normal intestinal changes. Other metamorphic changes, including breakdown of the intestinal epithelium, are inhibited. According to a Russian paper (Dyban, *et al.*, 1960)[167], holoblastic metamorphosis (cleavage) of the frog embryo is inhibited by sodium

perchlorate at the 100-125 mg/l level (70-90 mg/l as ClO_4), but not below this. The acute toxic level of sodium perchlorate to tadpoles (unspecified) is said to be 2000 mg/l for 24 hours (Durand, 1938)[168].

Other studies, not directly related to environmental problems, concern the effect of perchlorate on isolated heart muscle (Eichler, 1929)[169], sartorius (thigh) muscle (Boehm, 1929)[170], transversely striated muscle (Messini, 1929)[171], and toe muscles (Foulks, *et al.*, 1973)[172] of the frog.

Echinoderms. Lallier (1957)[173] has observed that embryonic development of the sea urchin, *Paracentrotus lividus*, is modified by treatment of the eggs 1/2 hour after fertilization with seawater containing 5000 mg/l or more of sodium perchlorate. Treatment of the unfertilized eggs for 20 hours with 15,000-30,000 mg/l of sodium perchlorate before but not after fertilization did not affect normal development. No evidence has been presented that moderate (500 mg/l) concentrations of perchlorate have any measurable effect on sea urchin embryos.

Annelids. Leeches (unspecified) are killed by a 2% solution of sodium perchlorate, but live several days in a 1% solution (Durand, 1938)[168].

Arthropods. No communications concerning the effect of perchlorate on freshwater or marine arthropods have been retrieved. A Russian paper (Rapoport, *et al.*, 1970)[174] reports a high incidence of phenocopial events in *Drosophila melanogaster* (fruit fly) exposed to "sublethal" levels of sodium perchlorate (*ca.* 2.5×10^{-5}M or 2.5 mg/l as ClO_4). (A phenocopial event is an embryonic change resembling a mutation and brought about by an agent such as a chemical.) This represents by far the greatest degree of sensitivity to perchlorate of any animal tested, and clearly merits verification.

Plants. There are no perchlorate toxicity studies relating to aquatic flowering plants. However, Weaver (1942)[175] found that Biloxi soybeans grown in water culture exhibit toxic symptoms (crumpling of leaves) at perchlorate level as low as 2.5 mg/l four days after application. Soybeans grown in sand were noticeably less susceptible.

According to Klemperer (1957)[176], uptake of iodide (^{131}I) by the seaweed *Fucus cerenoides* is competitively inhibited by perchlorate at the 0.1 mg/l level in seawater.

Microorganisms. Two papers report bacterial growth inhibition. Development of *Clostridium tyrobutyricum* in glucose broth is inhibited by 1000-2000 mg/l of potassium perchlorate (Vos, 1949)[177]. Durand (1939)[168] states that a 2% solution of sodium perchlorate is required to check the growth of *E. coli*, and about 10% to check the growth of *Staphylococcus aureus* and *Sterigmatocystic nigra*. Of seven unicellular algae, Greenhalgh and Riley (1961)[178] have reported that 1000 mg/l of perchlorate is without effect for *Phaeodactylum tricornutum, Monochrysis lutheri, Isochrysis galbana,* and *Nanochloris oculata*. Some inhibition of growth at this level was exhibited by *Prorocentrum micans, Gymnodinium vitiligo,* and *Peridinium trochoideum*.

Biotransformation and Subcellular Physiology. A few microorganisms are able to metabolize perchlorate; most are not. Tromballa and Broda (1971)[179] have shown that perchlorate is absorbed by the alga *Chlorella fusca* only to a small extent, and is not reduced to chloride. Hackenthal, *et al.* (1964, 1966)[180 181], on the other hand, have found that perchlorate (10^{-2}M) is reduced to chloride by several species of heterotrophic bacteria, including the very common *E. coli, Pseudomonas aeruginosa,* and *Serratia marcescens*, as well as several *Staphylococcus, Micrococcus* and *Sarcina sp.* Some *Streptococcus* and *Flavobacterium spp.* were inactive. The authors performed a number of experiments in competitive reduction of perchlorate and nitrate which appear to demonstrate that the two anions are substrates of the same enzyme, nitrate reductase.

Perchlorate has been shown to inhibit a few enzyme systems. Blaschko (1935)[182] has reported that the decomposition of hydrogen peroxide by horse liver catalase is reversibly inhibited by more than 90% during the first 5 minutes by 10^{-4}M (10 mg/l) perchlorate. Oxalacetic decarboxylase isolated from *Cucurbita pepo* by Byerrum, *et al.* (1950)[183] was inhibited more than 90% by 0.007M sodium perchlorate.

Some viruses are inactivated by perchlorate (Freifelder, 1966)[184]. On exposure to 0.1-5M sodium perchlorate, many bacteriophages eject their DNA intact.

Analytical Chemistry of Perchlorate in Water. Although many papers concerning analysis for perchlorate have appeared in the last two decades, only a few describe procedures suitable for estimation of perchlorate in natural waters and wastewaters, i.e., at the mg/l level.

Nabar and Ramachandran (1959)[185] have reviewed earlier methods, most of which involved reduction of perchlorate to chloride by means of

a reagent such as sulfur (Durand, 1937)[186], amalgamated zinc (Haight, 1953)[187], titanium (III) chloride (Eagles, 1954)[188], or vanadium (III) sulfate (Zatko and Kratochvil, 1965)[189]. The chloride produced is determined volumetrically by the usual methods, or the excess reducing agent may be estimated. Because the reduction usually proceeds well only in concentrated sulfuric acid solution, a substantial dilution of the original sample is inevitable. This contributes to the low precision of the method, which is no better than ± 20 mg/l. In a procedure by Trautwein and Guyon (1968)[190], perchlorate is reduced with tin (II) chloride, and the excess tin is determined spectrophotometrically. The best precision is in the order of ± 1 mg/l.

The perchlorate ion-selective electrode based on liquid ion exchange extraction has received the most recent attention (Baczuk and DuBois, 1968; Coetzee and Freiser, 1968, 1969; Manahan, *et al.*, 1970; Covington and Thain, 1971; Ishibashi and Kohara, 1971; James, *et al.*, 1972; Rohm and Guilbault, 1974)[191,192,193,194,195,196,197,198]. A concentration cell (Figure 4) is set up with a liquid ion exchange membrane between the test and reference solutions. The membrane typically consists of a substituted phenanthroline metal chelate in nitrobenzene or high molecular weight quaternary ammonium salts in 1 decanol. Coetzee and Freiser (1969)[193] quote a useful range of 10^{-1} to 10^{-4}M (10-10,000 mg/l); more recently Ishibashi and Kohara (1971)[196] have reported precision in the 1 mg/l range. Higher sensitivity may also be achieved by precipitation titration of the perchlorate with tetraphenylarsonium chloride using the ion-selective electrode as an indicator.

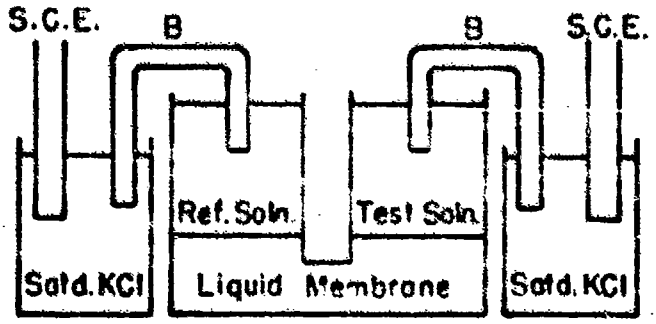


Figure 4. Concentration cell for perchlorate (Ishibashi and Kohara, 1971)[196]
(S.C.E. = Saturated Calomel Electrode; B=Salt bridge, 30% NH_4Cl)

A large number of procedures have been devised whereby a dye is added to the solution containing perchlorate, and the perchlorate-dye complex is selectively extracted into an appropriate organic solvent. The complex is then determined spectrophotometrically. These procedures, which are summarized in Table 14, give sensitivities on the order of 0.1 mg/l or better, but may be subject to interference by many ions, including those in common detergents. In a related procedure, the solution containing perchlorate is equilibrated with a colored liquid ion exchanger consisting of a quaternary ammonium erdmanate in xylene-hexone (Clifford and Irving, 1964; Irving and Damodaran, 1965)[199,200], the tetraphenylarsonium salt of eosin or bromothymol blue in chloroform (Tribalat and Piolet, 1961)[201], or the quaternary salt of dimethylprotriptyline in chloroform-cyclohexanealcohol (Borg, 1969)[202]. The perchlorate ion displaces a colored anion into the aqueous phase, where it is determined spectrophotometrically. The useful range is 1-10 mg/l. In yet another variation (Shigematsu, *et al.*, 1970)[203] perchlorate is extracted into nitrobenzene as the ^{65}Zn-o-phenanthroline complex, which is determined by means of a scintillation counter. The useful range extends down to 0.02 mg/l.

A gravimetric procedure using tetraphenyl-phosphonium ion to precipitate the perchlorate is sensitive in the 1 mg/l range (Willard and Perkins, 1953)[204]. Perchlorate has also been determined gravimetrically as the potassium salt by isotopic dilution with $^{36}KClO_4$, with a precision on the order of $\pm$ 10 mg/l (Johannesson, 1962)[205].

An infrared technique using potassium bromide discs is said to be accurate in the range of 10 mg/l perchlorate (Greenhalgh and Riley, 1960, 1961; Loach, 1972)[206,178,207].

Environmental Prevalence of Perchlorate. In 1958, Baas Becking, *et al.*[221] reported that perchlorate was present in concentrations as high as 1000 mg/l in the rivers, estuaries and coastal waters of Australia. Others (Greenhalgh and Riley, 1960, 1961; Loach, 1962; Iwasaki, *et al.*, 1963)[206,178,207,208] have attributed these findings to errors in the experimental procedure, and have reported finding no detectable perchlorate in natural water samples. Loach has reported finding 9-12 mg/kg of perchlorate (as $KClO_4$) in garden vegetables and 21-28 mg/kg in urine. Weiss and Stanbury (1972)[220], on the other hand, report finding no perchlorate in urine or serum to a detection limit of 5 mg/kg. There may have been a significant difference in samples, but it seems more likely that the difference was in analytical procedures.

Table 14. Spectrophotometric Determination of Perchlorate

Color Agent	Solvent	λ_{max} of Complex nm	Sensitivity Claimed ±mg/l	Reference
Methylene blue	dichloroethane	655	.05 - 2	Iwasaki, *et al.*, (1963)[208]
Crystal violet	chlorobenzene	590	.01	Uchikawa (1967)[209]
Crystal violet	benzene	615	.1	Hedrick & Berger (1966)[210]
Nile blue	1,2-dichlorobenzene	650	.5	Savic & Savic (1969)[211]
Brilliant green	benzene	640	≤1	Fogg, *et al.* (1971)[212]
Brilliant green	benzene	619	.5	Golosnitskaya and Petrashen (1964)[213]
Malachite green	benzene	640	.5	Golosnitskaya and Petrashen (1964)[214]
Neutral red	nitrobenzene	---	.16	Tsubouchi and Yamamoto (1970)[215]
Ferroin	n-butyronitrile	510	.01	Fritz, *et al.* (1964)[216]
1,10-Phenanthroline-Iron (II) chelate	nitrobenzene	516	.1	Yamamoto, *et al.* (1964)[217]
Cuproin-Copper (I) chelate	chloroform or chlorobenzene	550	2	Yamamoto, *et al.* (1969)[218]
2,2'-Dipyridyl-Iron (II) chelate	nitrobenzene	524	.5	Yamamoto and Kotsuji (1964)[219]
Neocuproin-Copper (II)	ethylacetate	456	5 - 10*	Weiss and Stanbury (1972)[220]

*Biological fluid.

Summary and Conclusions. Although few definitive studies have been conducted, it appears that the acute toxicity of perchlorate ion to aquatic animals and microorganisms is very low, with toxic levels probably exceeding 1000 mg/l for periods of 24 hours and longer. Fish, leeches, and tadpoles all survive indefinitely in water with perchlorate levels above 500 mg/l. Because perchlorate is an important anti-thyroid agent, however, chronic effects may appear at much lower levels. Newts maintained at a level of 36 mg/l exhibit significant histological changes in the thyroid and pituitary, and continuous exposure to 360 mg/l results in arrested metamorphosis in tadpoles and a grossly enlarged thyroid in guppies. Holoblastic metamorphosis of frog embryos is reported to be inhibited at 70-90 mg/l perchlorate.

Growth of some bacteria and unicellular algae has been shown to be similarly insensitive to perchlorate, and some common bacteria are capable of metabolizing perchlorate. No aquatic organism has been found to be affected by perchlorate in the mg/l range, although soybean plants grown in water culture show toxic symptoms at 2.5 mg/l. While studies to establish minimum chronic levels for aquatic organisms would be desirable, perchlorate does not seem likely to become an environmental hazard.

Recommendations for Further Work.

1. Acute Toxicities. The acute, 96-hour median tolerance limit for potassium perchlorate should be determined for a native freshwater fish, such as the rainbow trout (*Salmo gairdneri*), brook trout (*Salvelius fontinalis*), or fathead minnow (*Pimephales promelas*).

The acute, 96-hour median tolerance limit for potassium perchlorate should be determined for a native saltwater fish. We recommend the common killifish (*Fundulus heteroclitus*) or the sheepshead minnow (*Cyprinodon variegatus*). To estimate the toxicity of perchlorate to native arthropods, the immobilization level should be determined in freshwater for *Daphnia magna*. For each animal studied, the effect of temperature on perchlorate toxicity should be examined.

2. Chronic Toxicities. A complete chronic bioassay should be conducted with the same fish used in the acute tests, at perchlorate levels of 1 and 10 mg/l, and at several temperatures. This test will be concerned with the viability of the eggs, survival and growth of the fry, and sexual maturity of the adults.

3. Some common aquatic flowering plants should be maintained in the effluent water from the chronic assays to test the phytotoxicity of perchlorate.

STRONTIUM SALTS

Approach to Toxicity to Aquatic Organisms. The published literature has been surveyed through July 1974 by means of *Chemical Abstracts*. Citations for all strontium compounds were reviewed; however, abstracts of articles pertaining to radioisotopes of strontium were retrieved only insofar as they related to uptake and distribution of nonradioactive strontium. For Volumes 1-78 (1907-1973), the appropriate index headings were surveyed; for Volumes 79-80 (1973-1974), the keyword citations were scanned issue by issue.

Additional references were recovered from secondary sources. Of particular value were the reviews of Ellis (1937)[222], Doudoroff and Katz (1953)[223], and McKee and Wolf (1963)[224].

Toxicity to Aquatic Organisms.

Fish. The first published study of the toxicity of soluble strontium to fish was conducted in 1881 by Richet[225], who found that the survival of a number of small Mediterranean fishes exceeded 48 hours on exposure to 2.2 g/l of strontium as strontium chloride. Subsequently, the low order of toxicity of strontium has been confirmed with freshwater fishes. Jones (1939, 1957, 1964; Murdock, 1953)[226,227,228,229] showed that the minimum lethal concentration for sticklebacks (i.e., survival = survival of controls = 10 days) is 1200 mg/l of strontium as strontium nitrate, or 13.7 mM. By comparison, the minimum lethal concentration of calcium nitrate is 20 mM; hence, the toxicity of strontium is only slightly greater than that of calcium to sticklebacks. Earlier studies by Powers (1917)[230] with crucian goldfish and by Imao (1932)[231] with small freshwater fish, *Oryzias latipes*, are in close agreement with the work of Jones. Acute toxicity data for fishes are summarized in Table 15. Clearly, no discharge of strontium from pyrotechnic loading facilities or any other likely source could reasonably be considered an environmental hazard.

Concerning the toxicity of strontium to fish embryos, Mathews (1904)[232] has noted that 2/3 n $SrCl_2$ (presumably 29.2 g Sr/l) is the least concentration preventing formation of embryos in the eggs of the common killifish (*Fundulus heteroclitus*) at 20°C.

Amphibia. A single paper by Pincussen (1927)[233] states that 0.1 n strontium chloride (presumably 4380 mg Sr/l) is lethal to 4 of 10 *Bufo*

Table 15. Toxicity of Strontium to Freshwater and Marine Fishes

Fish	Salt	Sr Concn. mg/l	Reported Effect	Reference
Goldfish (*Carassius carassius*)	$SrCl_2$	8,500	Lethal in 17-31 hr	Powers (1917)[230]
Goldfish (*Carassius carassius*)	$Sr(NO_3)_2$	3,980	Lethal in 32-146 hr	Powers (1917)[230]
Goldfish			Depression of movement and respiration	Okuda (1932)[234]
Stickleback (*Gasterosteus aculeatus*)	$Sr(NO_3)_2$	1,200	Min. lethal conc.	Jones (1939)[226]
Stickleback (*Gasterosteus aculeatus*)	$Sr(NO_3)_2$	3,000	Av. survival 96 hr	Jones (1939)[226]
Stickleback (*Gasterosteus aculeatus*)	$Sr(NO_3)_2$	7,000	Av. survival 48 hr	Jones (1939)[226]
Stickleback (*Gasterosteus aculeatus*)	$Sr(NO_3)_2$	10,000	Av. survival 24 hr	Jones (1939)[226]
Oryzias latipes	$SrCl_2$	11,000	Lethal in 24 hr	Iwao (1936)[231]
Mediterranean Fishes				
Serranus cabrilla, *Crenolabrus Mediterraneus*, *Julius vulgaris*, *Julius giofradi*	$SrCl_2$	2,200	Survive > 48 hr	Richet (1881)[225]

tadpoles in one hour and 9 of 10 in 20 hours, when the tadpoles are exposed to intermittant light. Nearly all tadpoles maintained in the dark survive at this concentration.

Invertebrates. Several studies have shown the microcrustacean, *Daphnia magna*, to be substantially more sensitive to strontium salts than fish. Thus Anderson (1948)[235] found the 64-hour immobilization threshold for strontium chloride to be 63 mg/l as Sr, while Bringmann and Kuhn (1959)[37] reported the 48-hour toxic threshold to be 210 mg/l. In the most recent study, Biesinger and Christensen (1972)[236] have generally confirmed the acute toxicities documented in the earlier works. In addition, they conducted chronic studies over a period of three weeks, which indicated that reproduction of *Daphnia* is impaired by 16% at a level of 42 mg Sr/l and by 50% at a level of 60 mg/l. Some growth parameters were also measured; there is an average 24% loss in weight on exposure for 3 weeks at 100 mg/l. Anderson reported that strontium was much more toxic to *Daphnia* than calcium on a molar basis, relative to fishes, while Biesinger and Christensen found that the difference became significant only in chronic tests, i.e., during 21 days. Anderson's value for strontium appears low. It should be noted in any event that daphnids are probably most vulnerable during molting, and all animals will have undergone one or more molts in the course of each of these investigations.

For the planarian, *Polycelis nigra*, Jones (1940)[237] has found the threshold (48 hr) survival for strontium chloride to be 6600 mg/l as Sr, and for strontium nitrate, 3500 mg/l as Sr. On a molar basis, the toxicity is only slightly greater than for the corresponding calcium salts. Apparently nitrate is of greater concern to *Polycelis* than the cation. Acute toxicity data for invertebrates are summarized in Table 16.

Microorganisms. The few investigations carried out with protozoans indicate that the sensitivity to strontium salts is about the same as for *Daphnids* (Dale, 1913; Bringmann and Kuhn, 1959)[238,40]. Yarbrough and O'Kelley (1962)[239] have additionally shown that complete substitution of strontium for calcium at 0-2 mM (18 mg Sr/l) in the nutrient medium of *Paramecium multimicronucleatum* has no effect on the rate of growth. There is an important increase in the number of "avoidance reactions" exhibited by the animal, but the authors suggest that this is a calcium deficiency symptom rather than a strontium toxicity reaction. Some toxicity data are presented in Table 17.

For a wide range of bacterial species, strontium salts have been found to be inhibitory only at very high concentrations, as shown in

Table 16. Toxicity of Strontium to Invertebrates

Animal	Salt	Sr Concn. mg/l	Reported Effect	Reference
Daphnia magna	$SrCl_2$-$6H_2O$	210	48 hr Toxic threshold	Bringmann and Kuhn (1959)[37]
Daphnia magna	$SrCl_2$	63	64 hr Toxic threshold	Anderson (1948)[235]
Daphnia magna	$SrCl_2$-$6H_2O$	125	48 hr LC50	Biesinger & Christensen (1972)[236]
Daphnia magna	$SrCl_2$-$6H_2O$	86	21 day LC50	Biesinger & Christensen (1972)[236]
Polycelis nigra	$SrCl_2$	6,600	48 hr Toxic threshold	Jones (1940)[237]
Polycelis nigra	$Sr(NO_3)_2$	3,500	48 hr Toxic threshold	Jones (1940)[237]

Table 17. Toxicity of Strontium to Microorganisms

Microorganism	Salt	Sr Concn. mg/l	Reported Effect	Reference
Protozoa				
Microregma heterostoma	$SrCl_2$	329	28 hr toxic threshold for feeding	Bringmann and Kuhn (1959)[40]
Paramecium	$SrCl_2$	8000	All dead in 4.5 hr	Dale (1913)[238]
Bacteria				
Bacillus anthracis	$SrCl_2$	23×10^3	Growth inhibition threshold	Eisenberg (1919)[240]
Bacillus anthracis	$SrCl_2$	26×10^3	Growth inhibition	Eisenberg (1919)[240]
Sarcina tetragena	$SrCl_2$	17×10^3	Growth inhibition threshold	Koulumies (1946)[242]
Micrococcus phogenes	$SrCl_2$	17×10^3	Growth inhibition threshold	Eisenberg (1919)[240]
Micrococcus candicans	$SrCl_2$	13×10^3	Growth inhibition threshold	Eisenberg (1919)[240]
Corynebacterium diphtheriae	$SrCl_2$	6700	Growth inhibition threshold	Eisenberg (1919)[240]
Corynebacterium diphtheriae	$SrCl_2$	10×10^3	Growth inhibition	Koulumies (1946)[242]
Corynebacterium diphtheriae	$SrCl_2$	10×10^3	Growth inhibition threshold	Eisenberg (1919)[240]
Corynebacterium pseudodiphtheriae	$SrCl_2$	4×10^3	Growth inhibition	Koulumies (1946)[242]
Corynebacterium pseudodiphtheriae	$SrCl_2$	30×10^3	Growth inhibition threshold	Eisenberg (1919)[240]
Bacillus typhi	$SrCl_2$	13×10^3	Growth inhibition threshold	Eisenberg (1919)[240]
Bacillus pneumoniae	$SrCl_2$	10×10^3	Growth inhibition threshold	Eisenberg (1919)[240]
Bacillus pyocyaneus	$SrCl_2$	27×10^3	Growth inhibition threshold	Eisenberg (1919)[240]
Bacillus vulgare	$SrCl_2$			

Table 17. Toxicity of Strontium to Microorganisms (Continued)

Microorganism	Salt	Sr Concn. mg/l	Reported Effect	Reference
Vibrio cholerae	$SrCl_2$	1700	Growth inhibition threshold	Eisenberg (1919)[240]
Escherichia coli	$SrCl_2$	27×10^3	Growth inhibition threshold	Eisenberg (1919)[240]
Escherichia coli	$SrCl_2$	22×10^3	Growth inhibition threshold	Hotchkiss (1923)[241]
Escherichia coli	$SrCl_2$	18×10^3	Growth inhibition	Koulumies (1946)[242]
Salmonella paratyphi B	$SrCl_2$	10×10^3	Growth inhibition	Koulumies (1946)[242]
Salmonella paratyphi A	$SrCl_2$	10×10^3	Growth inhibition	Koulumies (1946)[242]
Eberthella typhosa	$SrCl_2$	18×10^3	Growth inhibition	Koulumies (1946)[242]
Proteus vulgaris	$SrCl_2$	18×10^3	Growth inhibition	Koulumies (1946)[242]
Shigella paradysenteriae	$SrCl_2$	7×10^3	Growth inhibition	Koulumies (1946)[242]
Brucella abortus	$SrCl_2$	7×10^3	Growth inhibition	Koulumies (1946)[242]
Pasteurella pestis (Tjevidei)	$SrCl_2$	7×10^3	Growth inhibition	Koulumies (1946)[242]
Pasteurella pestis (Soemsdang)	$SrCl_2$	22×10^3	Growth inhibition	Koulumies (1946)[242]
Hemophilus pertussis	$SrCl_2$	10×10^3	Growth inhibition	Koulumies (1946)[242]
Hemophilus influenza	$SrCl_2$	10×10^3	Growth inhibition	Koulumies (1946)[242]
Neisseria catarrhalis	$SrCl_2$	18×10^3	Growth inhibition	Koulumies (1946)[242]
Neisseria gonorrhoeae	$SrCl_2$	10×10^3	Growth inhibition	Koulumies (1946)[242]

Table 17. Toxicity of Strontium to Microorganisms (Continued)

Microorganism	Salt	Sr Concn. mg/l	Reported Effect	Reference
Staphylococcus aureus	$SrCl_2$	10 X 10^3	Growth inhibition	Koulumies (1946)[242]
Staphylococcus albus	$SrCl_2$	10 X 10^3	Growth inhibition	Koulumies (1946)[242]
Streptococcus pyrogenes	$SrCl_2$	10 X 10^3	Growth inhibition	Koulumies (1946)[242]
Streptococcus viridans	$SrCl_2$	10 X 10^3	Growth inhibition	Koulumies (1946)[242]
Diplococcus pneumoniae	$SrCl_2$	10 X 10^3	Growth inhibition	Koulumies (1946)[242]
Actinomyces graminis	$SrCl_2$	44 X 10^3	Growth inhibition	Koulumies (1946)[242]
Mycobacterium tuberculosis hominis	$SrCl_2$	7 X 10^3	Growth inhibition	Koulumies (1946)[242]
Mycobacterium tuberculosis bovis	$SrCl_2$	7 X 10^3	Growth inhibition	Koulumies (1946)[242]
Clostridium (7 species)	$SrCl_2$	7 to 10 X 10^3	Growth inhibition	Koulumies (1946)[242]
Leptospira icterohaemorrhagiae (3 strains)	$SrCl_2$	7 to 10 X 10^3	Growth inhibition	Koulumies (1946)[242]
Fungi				
Epidermophyton Kaufmann-Wolff	$SrCl_2$	26 X 10^3	Growth inhibition	Koulumies (1946)[242]
Trichophyton (3 species)	$SrCl_2$	26 to 52 X 10^3	Growth inhibition	Koulumies (1946)[242]
Penicillium (Orion)	$SrCl_2$	70 X 10^3	Growth inhibition	Koulumies (1946)[242]
Mucor corymbifer	$SrCl_2$	26 X 10^3	Growth inhibition	Koulumies (1946)[242]

Table 17. Toxicity of Strontium to Microorganisms (Continued)

Microorganism	Salt	Sr Concn. mg/l	Reported Effect	Reference
Cladisporium mansoni	$SrCl_2$	44 X 10^3	Growth inhibition	Koulumies (1946)[242]
Scopularia brevicaulis	$SrCl_2$	66 X 10^3	Growth inhibition	Koulumies (1946)[242]
Aspergillus fumigatus	$SrCl_2$	70 X 10^3	Growth inhibition	Koulumies (1946)[242]
Altenaria	$SrCl_2$	66 X 10^3	Growth inhibition	Koulumies (1946)[242]
Altenaria tenuis	$Sr(NO_3)_2$	30 X 10^3	ED50, germination inhibition	Somers (1959; 1961)[245 246]
Botrytis fabae	$Sr(NO_3)_2$	13 X 10^3	ED50, germination inhibition	Somers (1961)[246]
Algae				
Coccomyxa pringsheimii	-----	5 - 10	Inhibition of growth and calcium utilization	Walker (1956)[249]
Chlorella vulgaris	$SrCl_2$-$6H_2O$	>13 X 10^3	Lowest inhibitory conc.	den Dooren de Jong (1965)[248]

Table 17 (Eisenberg, 1919; Hotchkiss, 1923; Koulumies, 1946)[240,241,242]. Relative to calcium, the toxicity of strontium is not uniform; for some species it is of equal toxicity on a molar basis, while for others strontium is nearly ten times more inhibitory. At a low environmental level (1-10 mg/l) calcium is essential for growth and nitrogen fixation in a number of *Azotobacter species* (Norris and Jensen, 1957 & 1958)[243 244]. In each case it has been shown that strontium can substitute for calcium on an equimolar basis (2-20 mg Sr/l), thus indicating a nutritional function for strontium.

For a number of fungi, also listed in Table 17, the resistance to strontium is the same or greater than for bacteria (Koulumies, 1946; Somers, 1959 & 1961)[242,245,246]. For slime molds (Myxomycetes), the toxicity of strontium is said to be intermediate between that of magnesium, and barium the most toxic (Seifriz, 1949)[247].

The green algae exhibit very large specific variations in response to strontium. The lowest inhibitory concentration for *Chlorella vulgaris* exceeds 13 g/l (den Dooren de Jong, 1965)[248]. Strontium can substitute for calcium in the nutrition of *Chlorella pyrenoidosa* (Walker, 1953)[249], and as the chloride (but not the nitrate) it is largely without effect on the growth of *Stichococcus bacillaris* at 8.8 g Sr/l (Schroder, 1954)[250]. In contrast, calcium utilization by *Coccomyxa pringsheimii* is inhibited by strontium at a level of 1 to 5 mg/l, and in a low calcium environment (2-6 mg/l), growth is strongly inhibited by strontium at 5-10 mg/l (Walker, 1956)[251]. For *Protosiphon botryoides*, substitution of strontium for calcium in the nutrient media has the curious property of preventing formation of motile cells (zoospores), with partial inhibition of growth (O'Kelley and Herndon, 1959)[252]. It has also been noted that some green algae, including *P. botryoides* and *C. pyrenoidosa*, produce larger, more highly vacuolated cells in strontium replacement media (Moss, *et al.*, 1971)[253].

Strontium in the Aquatic Environment. Although not a major element in the aquatic environment, strontium is by no means rare. The concentration in sea water has been variously measured at 8.1-10 mg/l (Black and Mitchell, 1952; Bowen, 1956; Eremeeva, *et al.*, 1972)[254,255,256], while the freshwater concentration usually falls in the range of 0.05 to 0.5 mg/l (Templeton and Brown, 1964)[257], though occasionally exceeding 5 mg/l (Curtis and Stueber, 1973)[258]. The ratio of strontium to calcium is usually 1-5/1000 in freshwater[237] and 1/4 in seawater (Ophel and Fraser, 1970)[259]. Thus freshwaters high in calcium are usually relatively high in strontium and *vice versa*.

Because of the concern with accumulation of radiostrontium (^{85}Sr, ^{89}Sr, ^{90}Sr) in the environment, many investigations have been carried out on the accumulation of strontium in aquatic organisms and its movement up the food chain. The work concerning fish has been reviewed by Simmons (1971)[260]. Although there are important quantitative differences among species, it is apparently true that discrimination among freshwater plants (Owens, *et al.*, 1961)[261] and some fish[257, 260] and microorganisms (Kevern, 1964)[262] is higher in favor of calcium in high calcium waters and lower in very low calcium waters, indicating that many organisms will satisfy at least part of their calcium requirement with strontium if and only if calcium is in short supply. Where the calcium concentration is sufficiently high, on the other hand, discrimination is a matter of chemical equilibrium. The discrimination in favor of calcium tends to be higher in marine fishes than freshwater fishes. It may be that marine fishes, exposed to a Sr/Ca ration 100 times greater than freshwater fishes, have evolved a mechanism to avoid incorporating too much strontium. Discrimination is measured in terms of a discrimination factor, D (also designated the observed ration OR):

$$D = \frac{(Sr/Ca)_{\text{sample}}}{(Sr/Ca)_{\text{precursor}}}$$

For simple organisms, such as algae, "precursor" refers to the surrounding water; for higher animals, however, it could also refer to the food source. For fish there is evidence that discrimination is greater against ingested strontium than against strontium absorbed through the gills (Berg, 1969)[263]. However, one study showed large differences in observed ratios for different species of fish in the same lake (Ophel and Judd, 1967)[264]. The authors interpreted this as indicating different feeding habits of the fish.

Table 18 lists discrimination factors for a wide range of freshwater and marine organisms. Discrimination factors are generally greater than one for freshwater organisms and less than one for marine organisms, with some important exceptions. The marine brown algae discriminate in favor of strontium (Yamamoto, *et al.*, 1969)[265], while the freshwater plant *Nymphaea odorata* takes up almost no strontium[259]. Insofar as strontium is concentrated in the food chain, dietary preferences could be critical. There is, however, no evidence from Table 18 that bioconcentration is occurring.

Table 18. Discrimination Factors: $D = \frac{(Sr/Ca)\ organism}{(Sr/Ca)\ water}$

Organism	Water	D	Reference
Brown algae	Sea	4.2	Bowen (1956)[255]
Red algae	Sea	1.3	Bowen (1956)[255]
Green algae	Sea	.50	Bowen (1956)[255]
Chiton shells	Sea	.81	Bowen (1956)[255]
Cephalopod bone	Sea	.57	Bowen (1956)[255]
Other mollusc shells	Sea	.27	Bowen (1956)[255]
Corals	Sea	1.4	Bowen (1956)[255]
Brown trout bone	Fresh	.45-.56	Templeton & Brown (1964)[257]
Brown trout muscle	Fresh	.53-1.00	Templeton & Brown (1964)[257]
Algae:			
Spirogyra ssp.	Fresh	1.5	Ophel & Fraser (1970)[259]
Nitella sp.	Fresh	.6	Ophel & Fraser (1970)[259]
Mosses:			
Fontinalis sp.	Fresh	1.2	Ophel & Fraser (1970)[259]
Vascular plants:			
Typha angustifolia	Fresh	1.2	Ophel & Fraser (1970)[259]
Sparganium fluctuans	Fresh	1.0	Ophel & Fraser (1970)[259]
Potamogeton (spp)	Fresh	1.5-2.1	Ophel & Fraser (1970)[259]
Najas flexilis	Fresh	1.2	Ophel & Fraser (1970)[259]
Scirpus (2 spp)	Fresh	1.5-2.2	Ophel & Fraser (1970)[259]
Eriocaulon septangulare	Fresh	0.5	Ophel & Fraser (1970)[259]
Pontederia cordata	Fresh	1.1	Ophel & Fraser (1970)[259]
Juncus militaris	Fresh	1.6	Ophel & Fraser (1970)[259]
Nymphaea odorata	Fresh	.1	Ophel & Fraser (1970)[259]
Nuphar variegatum	Fresh	1.6	Ophel & Fraser (1970)[259]

Table 18. Discrimination Factors: $D = \frac{(Sr/Ca)\ organism}{(Sr/Ca)\ water}$ (Continued)

Organism	Water	D	Reference
Vascular plants (cont):			
Brasenia schreberi	Fresh	.4	Ophel & Fraser (1970)[259]
Ceratophyllus demersum	Fresh	2.2	Ophel & Fraser (1970)[259]
Utricularia vulgaris	Fresh	1.7	Ophel & Fraser (1970)[259]
Rainbow trout	Fresh	.37	Ichikawa (1960)[261]
Snail: *Lymnaea stagnalis*	Fresh	*ca*.5	van der Borght (1962)[267]
Seal: *Phoca* (2 spp)	Sea	.038-.14	Kostareva, *et al.* (1970)[268]
Salmon: *Oncorhynchus* (2 spp)	Sea	.22-.26	Kostareva, *et al.* (1970)[268]
Herring: *Clupea harengus*	Sea	.117-.15	Kostareva, *et al.* (1970)[268]
Flounder: *Lepidopsetta* sp.	Sea	.26	Kostareva, *et al.* (1970)[268]
Crustacean: *Daphnia magna*	Fresh	.58	Marshall, *et al.* (1962)[269]

Analysis of Strontium. Because of the very low order of toxicity of strontium compounds, no effort has been made to collect and review articles on trace analysis of strontium in water. Methods are described in Standard Methods for the Examination of Water and Wastewater (13th Edition) and in some references from this report[254,255,257].

Conclusions. The acute toxicity of aqueous strontium is very low, being only slightly greater than that of calcium for a wide variety of freshwater and marine organisms. Furthermore, there is no indication of bioaccumulation of strontium in the aquatic food chain. It is inconceivable that any likely wastewater discharge of nonradioactive strontium could present an environmental hazard. For this reason, no further toxicity studies would appear to be justified at this time.

SUMMARY OF RECOMMENDATIONS

In the preparation of these reports, many deficiencies in our knowledge of possible adverse effects of the various munitions on aquatic life soon became apparent. Accordingly, brief recommendations have been made concerning further research necessary to correct these deficiencies. These recommendations, which involve complex and prolonged chemical and biological investigations, would provide the basis for a major research program. However, with the exception of DNT, this work cannot be justified in terms of the likely environmental hazards presented by the wastewaters from the munitions concerned. The maximum discharge of most of these materials in munitions wastewaters is known to be exceedingly small, and fragmentary evidence indicates a low order of toxicity. Thus, it is not intended that the recommendations made in these reports should be taken uncritically as the basis for a "Request for Proposal". It is believed that the research proposed is desirable in terms of advancing our knowledge concerning the potential problems presented by munitions wastes. It is also believed that this work would be ideally suited for in-house research. The large number of compounds involved, the uncertainties concerning their physical and chemical behavior in water, and the probability that many of the compounds will merit no more than a few simple bioassays while others justify extended study. This will involve researchers in many day-to-day decisions concerning the direction of the work, which could be time-consuming and cumbersome where an outside contractor is involved.

The recommendations for further research work detailed for seven of the compounds in the report are summarized in Table 19. No toxicity studies are proposed for either red phosphorus or strontium compounds.

Table 19. Summary of the Recommendations for Further Work on Seven of the Waterborne Pollutants

Pollutant	Acute Toxicity Studies	Chronic Toxicity Studies	Microbial Degradation Studies	Wastewater Analysis Studies
DNT isomers and wastewaters	X*	X*†	X◊	X**
Tetryl, picric acid and picramic acid	X	X	X	X

Table 19. Summary of the Recommendations for Further Work on Seven of the Waterborne Pollutants (Continued)

Pollutants	Acute Toxicity Studies	Chronic Toxicity Studies	Microbial Degradation Studies	Wastewater Analysis Studies
Tetranitroresorcinol and Lead Styphnate	X	X	X	X**
PETN	X*	X*	X*	X*
Nitroguanidine	X*	X*	X	X††
Tetracene	X*	X*	X	X††
Potassium perchlorate‡‡	X	X		

*To include any important degradation products of the compounds.

†To include any possible accumulation in aquatic organisms.

‡To include especially the 2,4- and 2,6- isomers of DNT.

**To include identification of any photochemical degradation products.

††Analytical procedures are required to be developed for wastewaters.

‡‡Effects of wastewaters on aquatic flowering plants to be studied.

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APPENDIX A

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LIST OF ABBREVIATIONS

Ar	Aromatic
AW	Acid Washed
BOD	Biological Oxygen Demand
cm	centimeter
ca.	about
concn.	concentration
DNT	Dinitrotoluene
ED_{50}	Median Effective Dose
g	grams
GSH	Glutathione, reduced
GSSG	Glutathione, oxidized
glc	gas-liquid chromatography
hr	hours
i.r.	infrared
kg	kilograms
l	liters
lb	pounds
LC	Lethal Concentration
LD_{50}	Median Lethal Dose
M	Molar (solution)
mg	milligrams
m	meta
min	minutes
N	Normal (solution)
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced
ng	nanograms
nm	nanometers (10^{-9} meters)
NTIS	National Technical Information Service

o	ortho
p	para
PETN	Pentaerythritoltetranitrate
pH	Hydrogen Ion Concentration, minus log of
TDC	Thermal Conductivity Detector
TLm	Median Tolerance Limits
TSH	Thyroid Stimulating Hormone
tlc	thin-layer chromatography
u.v.	ultraviolet
µg	micrograms
°	degrees: °C, Centigrade Scale
II	Divalent
III	Trivalent
λ	wavelength
^{14}C	Carbon 14 isotope
^{131}I	Iodine 131 isotope
^{36}K	Potassium 36 isotope
^{63}Ni	Nickel 63 isotope
^{85}Sr, ^{89}Sr ^{90}Sr	Strontium 85, 89, 90 isotopes
^{65}Zn	Zinc 65 isotope